The Acute Cognitive, Psychological and Electrophysiological effects of Cannabis Constituents

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PhD

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Preface

This thesis is a “Thesis incorporating publications”. This implies that certain sections or chapters will be taken from published journal articles which I am the first author of. For instance, sections in the introduction are taken from my review article which has been published in Current Pharmaceutical Design (Englund et al., 2012), and the chapter “The cognitive and psychological effects of Δ9-THC and CBD” is an original article published in Journal of Psychopharmacology (Englund et al., 2013). As the entire chapter is made up of the published article, it will also be preceded and followed by additional comments. The various measurements used for the experiments will be presented in the Appendices section.

Table of content

Abstract	9-10
Introduction	11-32
Cognitive and Psychological effects of Δ9-THC and CBD	33-59
Electrophysiological effects of Δ9-THC and CBD	60-96
Cognitive and Psychological effects of Δ9-THC and Δ9-THCV	97-126
Summary and Discussion	127-134
Appendices	135-163
References	164-192

Table of figures

Box 1. Early medical use of cannabis in Asia and Africa

Box 2. Effects of cannabis intoxication among recreational users

Image 1 The three subspecies of the cannabis plant

Image 2 Glandular trichomes of the cannabis plant

Image 3 Cannabinoid biosynthesis

Image 4 Endocannabinoid signalling

Table 1 List of intravenous cannabinoid studies

Table 2.1 Time course of experimental day of study 1

Table 2.2 Sample characteristics of study 1

Figure 2.1 Plasma cannabinoid concentration of study 1

Figure 2.2 PANSS positive score of study 1

Table 2.3 Frequency of clinically significant psychosis of study 1

Figure 2.3 SSPS score of study 1

Table 2.4 Cognitive performance of study 1

Figure 2.4a + b Immediate and delayed verbal recall of study 1

Table 3.1 Experimental timeline for EEG recording of study 1

Table 3.2 Participant demographics of EEG analysis

Table 3.3 EEG electrode grouping

Figure 3.1 Location of EEG electrodes

Figure 3.2 Percentage correct responses on the N-back task

Figure 3.3 Reaction time on the N-back task

Figure 3.4 PANSS positive score for EEG analysis

Figure 3.5 SSPS score for EEG analysis

Figure 3.6. Alpha amplitude according to electrode location

Figure 3.7. Alpha amplitude change across sessions for each scalp location

Figure 3.8. Alpha amplitude across sessions for each group

Figure 3.9. Beta amplitude according to electrode location

Figure 3.10. Beta amplitude change across sessions for each scalp location

Figure 3.11. Beta amplitude across sessions for each group

Figure 3.12. Delta amplitude according to electrode location

Figure 3.13. Delta amplitude change across sessions for each scalp location

Figure 3.14. Delta amplitude across sessions for each group

Figure 3.15. Theta amplitude according to electrode location

Figure 3.16. Theta amplitude change across sessions for each scalp location

Figure 3.17. Theta amplitude across sessions for each group

Figure 3.18. Gamma amplitude according to electrode location

Figure 3.19. Gamma amplitude change across sessions for each scalp location

Figure 3.20. Gamma amplitude across sessions for each group

Figure 3.21. Bi-frontal Alpha coherence across sessions

Figure 3.22. Bi-frontal Theta coherence across sessions

Figure 4.1 Timeline of experimental weeks of study 2

Table 4.1. Participant demographics of study 2

Table 4.2 Effects of Δ9-THCV and placebo

Figure 4.2 A. Delayed verbal recall of study 2

Figure 4.2 B. Memory intrusions of study 2

Figure 4.3 Reverse digit span of study 2

Figure 4.4 A. CAPE positive symptoms of study 2

Figure 4.4 B. CAPE negative symptoms of study 2

Figure 4.5 A. UMACL hedonic tone of study 2

Figure 4.5 B. UMACL energetic arousal of study 2

Figure 4.5 C. UMACL tense arousal of study 2

Figure 4.6 Beck's anxiety inventory of study 2

Figure 4.7 A. VAS "anxious" of study 2

Figure 4.7 B. VAS "high" of study 2

Figure 4.7 C. VAS "stoned" of study 2

Figure 4.8 Heart rate of study 2

Figure 4.9 A. Plasma Δ9-THC and 11-OH-Δ9-THC concentration of study 2

Figure 4.9 B. Plasma Δ9-THCV and 11-OH-Δ9-THCV concentration of study 2

Figure 4.10 A. Subjective experience of pleasure of study 2

Figure 4.10 B. Subjective experience of Δ9-THC strength of study 2

Abstract

The cannabis available in many countries has been changing in recent years to favour a product increasingly high in its main component, the cannabinoid Δ9-THC. Epidemiological studies have highlighted that early, persistent and heavy cannabis use, particularly for cannabis high in Δ9-THC, is associated with an increased risk of development of schizophrenia. However, cannabis is a complex plant which also produces over 100 other cannabis compounds, each with a unique pharmacological profile. Cannabinoids such as CBD and Δ9-THCV, which are virtually absent from cannabis sold on the black market in the UK today, may interfere pharmacologically with Δ9-THC and therefore protect against the negative effects associated with cannabis use.

This thesis is comprised of two experimental studies in healthy volunteers where the cognitive, psychological and electrophysiological effects of Δ9-THC, CBD and Δ9-THCV are explored.

Study 1

In the first part of study 1, the psychological and cognitive effects of Δ9-THC and CBD were explored in a placebo-controlled, between-subjects design with 48 healthy volunteers. Pre-treatment with CBD (600mg, oral) significantly inhibited IV Δ9-THC-induced (1.5mg) paranoia and impairments to delayed verbal recall. Also, significantly fewer participants experienced clinically significant psychotic symptoms following IV Δ9-THC if pre-treated with CBD compared to placebo.

In the second part of study 1, the electrophysiological effects of Δ9-THC and CBD were explored in the same sample, although the data from three participants were excluded. I found that Δ9-THC significantly reduced theta amplitude and coherence, although these were not correlated with psychopathology or inhibited by CBD. However, Δ9-THC-induced increases of alpha and delta amplitude was significantly inhibited by CBD.

Study 2

The second study was a within-subject, placebo-controlled, cross-over design study (N=10), exploring the effects of IV Δ9-THC (1mg) and Δ9-THCV (10mg/day oral for 5 days) in healthy male volunteers with minimal past cannabis use. I found that Δ9-THC did not produce significant increases in paranoia or psychosis, or impairments to immediate verbal recall. Δ9-THCV significantly inhibited Δ9-THC-induced heart-rate increase and impairments to delayed verbal recall. Δ9-THCV on its own produced a significant improvement in reverse digit span, and a trend towards increased anxiety.

Together, these results highlight the important protective role that CBD and Δ9-THCV play in recreational cannabis use against the negative effects of Δ9-THC. It also raises the possibility that CBD and Δ9-THCV may hold therapeutic value for mental illness and cognitive impairment.

Introduction

Cannabis

"Weary with toil, I haste me to my bed,

The dear repose for limbs with travel tired;

But then begins a journey in my head,

To work my mind, when body's work's expired….."

- *William Shakespeare, Sonnet 27*

It has been argued that Shakespeare is referring to the use of cannabis in this sonnet, as well as in sonnet 76 where he makes references to "compounds strange" and "noted weed". This has been hypothesised as a study found traces of cannabis and cocaine in pipes dug up from his home in Stratford-upon-Avon (Thackeray et al., 2001). Although this claim has been criticised, the use of cannabis has been pervasive in recent and ancient history for its mind-altering, therapeutic and spiritual effects (Touw, 1981). The first archaeological evidence of cannabis used by humans was found in China roughly 6000 years ago (Li, 1973), which indicated its cultivation for use of fibres rather as a psychoactive substance. The early medicinal use of cannabis has been extensively documented throughout Asia and Africa (Zuardi, 2006) (See Box.1), including Egypt (Russo, 2007).

Box.1 Early medical use of cannabis in Asia and Africa (Zuardi et al. 2006)

Region	Time	Treatment	
China	2700 BC	Rheumatic pain	Disorders of the female reproductive system
		Constipation	Malaria
India	1000 BC	Analgesia	Anti-inflammatory
		Anti-convulsant	Anti-parasitic
		Hypnotic	Digestive
		Tranquilizer	Appetite stimulant
		Anesthetic	Diuretic
		Anti-biotic	Aphrodisiac
Assyria	900 BC	Depression	Kidney stones
		Swelling and bruises	"Female ailments"
		Impotence	"Annulment of witchcraft"
		Arthritis	
Arabia	1000 AD	Diuretic	"Cleaning of the brain"
		Digestive	Ear pain
		Anti-flatulent	
Africa	1400 AD	Snake-bite	Fever
		Childbirth	Blood poisoning
		Malaria	Anthrax
		Astma	Dysentery

Nowadays recreational cannabis use is widespread across the world with an estimated 125-203 million people between the ages of 15-64 having used it at least once in the last year (UNDOC, 2011). It is estimated that roughly 9% of people who experiment with cannabis go on to develop dependence (Hall and Degenhardt, 2014). The rates of dependence among daily users range between 25-50% (Coffey et al., 2003).

Cannabis is most commonly used by smoking the dried flowering tops of the cannabis plant, often together with tobacco in a rolled cigarette (aka. joint). However, cannabis may also be eaten by incorporating it into cooked or baked food items (usually in fat, as the active components are fat soluble). The effects of smoked cannabis come on after a few minutes and last for roughly 2-3 hours, while eaten cannabis can take up to 2 hours before the effects are felt and can last for up to 8 hours (Jones and Stone, 1970). Presently and historically, cannabis is used medicinally to relieve certain symptoms and for various spiritual purposes, although it is most commonly used recreationally for its pleasurable effects. Cannabis produces a myriad of effects on the user which will vary not only between people, but also between moods and settings. Common experiences from recreational use are listed in Box.2 (Tart, 1970).

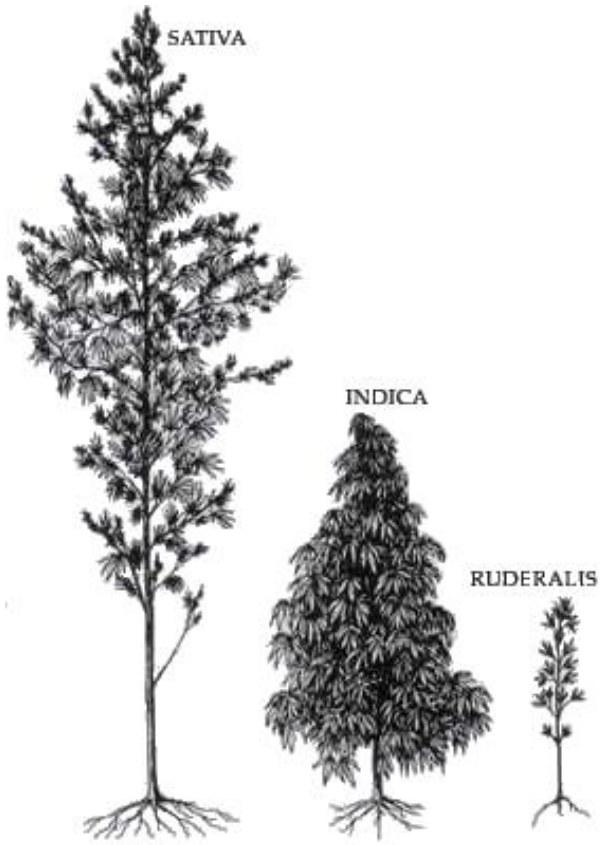
Box.2 Effects of cannabis intoxication among recreational users (Tart, 1970)

Sharpening of visual colours, shapes and textures
Enhanced sense of smell
Changes to sharpness and texture of sounds
Sensitivity to touch
Increased appetite
Tastes become enhanced
Time passing more slowly
Increased heart rate
Light-headed
Relaxation
Increased insightfulness
Less talkative
Enhanced sexual pleasure
Spontaneous laughter
Shortened memory span
Increased forgetfulness
Enhanced emotional awareness
Euphoria
Lowered inhibitions
Lose control of thoughts
Feeling axious or panic
Delusions
Grandiose thoughts and ideas
Spriritual effects
Paranoia and suspiciousness
Enhanced bodily awareness
Improved sleep
Impaired coordination
Feeling less/more sociable
Meaningless things find meaning

The plant

The cannabis plant is thought to originate from either Central Asian or the foothills of the Himalayan Mountains (Clarke and Watson, 2007) and is considered by some to be subdivided into 3 species: *Cannabis Sativa*, *Cannabis Indica* (aka. Afghanica), and *Cannabis ruderalis* (Hillig and Mahlberg, 2004). However, this is merely based on typology and morphology of the plants (see Image 1) rather than differences in the plants genetics or chemical composition, as most taxonomists consider cannabis to be monotypic (Harlan and de Wet, 1971; Small and Cronquist, 1976). The chemical compounds specific to the cannabis plant are known as cannabinoids, and the most common among them include Δ9-tetrahydrocannabinol (Δ9-THC), Δ9-tetrahydrocannabivarin (Δ9-THCV), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), cannabichromene (CBC) amongst others. It is estimated that cannabis contains over 108 different cannabinoids, along with roughly 400 other compounds such as terpenoids and flavonoids (Hanus, 2009).

Image 1. The three typological divisions of the cannabis plant, adapted from (Wikipedia, n.d.)



These chemical compounds are produced in glandular trichomes, mostly around the flowering tops of the plant and, to a far lesser extent, the leaves (Clarke and Watson, 2007) (see Image 2). Within the heads of these resin glands, the cannabinoids are stored within vesicles and protected from oxidative and enzymatic degradation by the glands waxy outer layer. The trichomes are thought to protect the plant against cold winds (Mahlberg et al., 1984), reflecting both infrared and ultraviolet rays (hence cooling the plant and reducing sunburn) (Roberecht et al., 1980), and protecting against UV radiation (Rhoades, 1977). Furthermore, bitter sesquiterpenes in the trichomes are thought to repel herbivores and their sticky and glutinous composition trap insects which prevent them from feeding and colonizing of the plant (Potter, 2009). The cannabinoids make up more than 80% of the trichomes and can sometimes make up 30% of the total weight of the flowering tops when dried (Russo, 2007; Clarke and Watson, 2007). Δ9-THC, CBD and CBC are produced from the same precursor CBG, each by means of a separate enzymes (Taura et al., 1995, 1996). The ratios of these enzymes also determine the ratio of cannabinoids the plant produces, and is

determined by a single allele: T, D and C (see Image 3). T and D alleles are co-dominant and depending on the genetic makeup of the plant, it will produce either a Δ9-THC dominant plant, a CBD dominant plant, or a plant with roughly equal levels of Δ9-THC and CBD.

Image 2. Glandular trichome of the cannabis plant, adapted from (Potter, 2013)

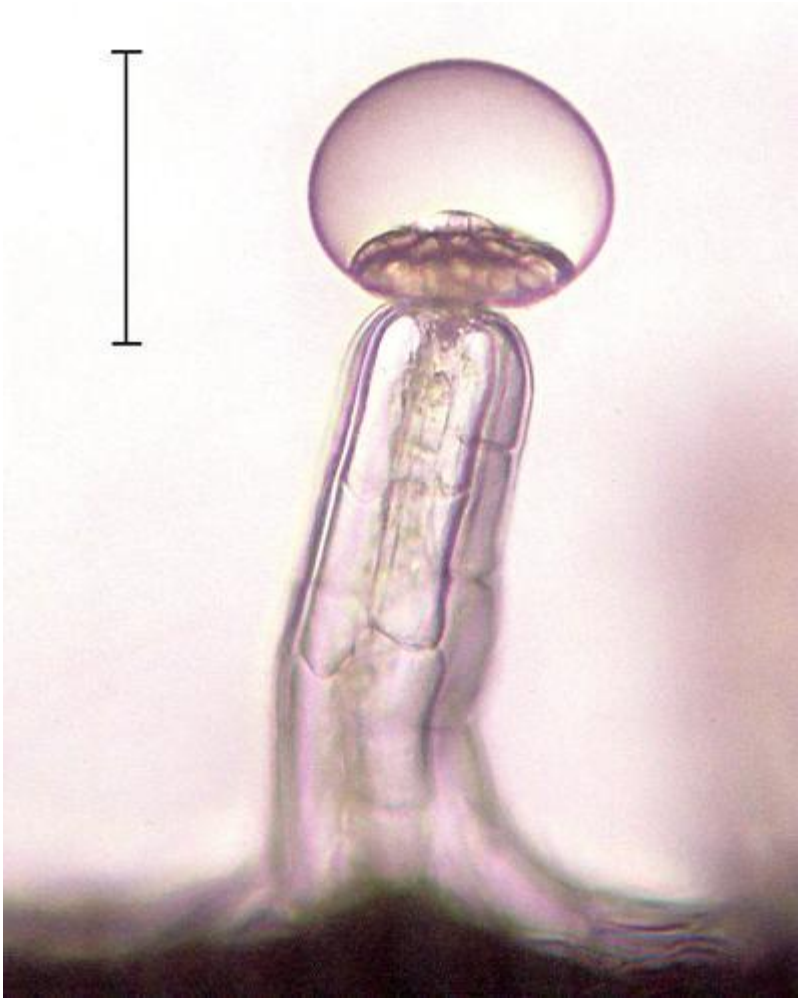
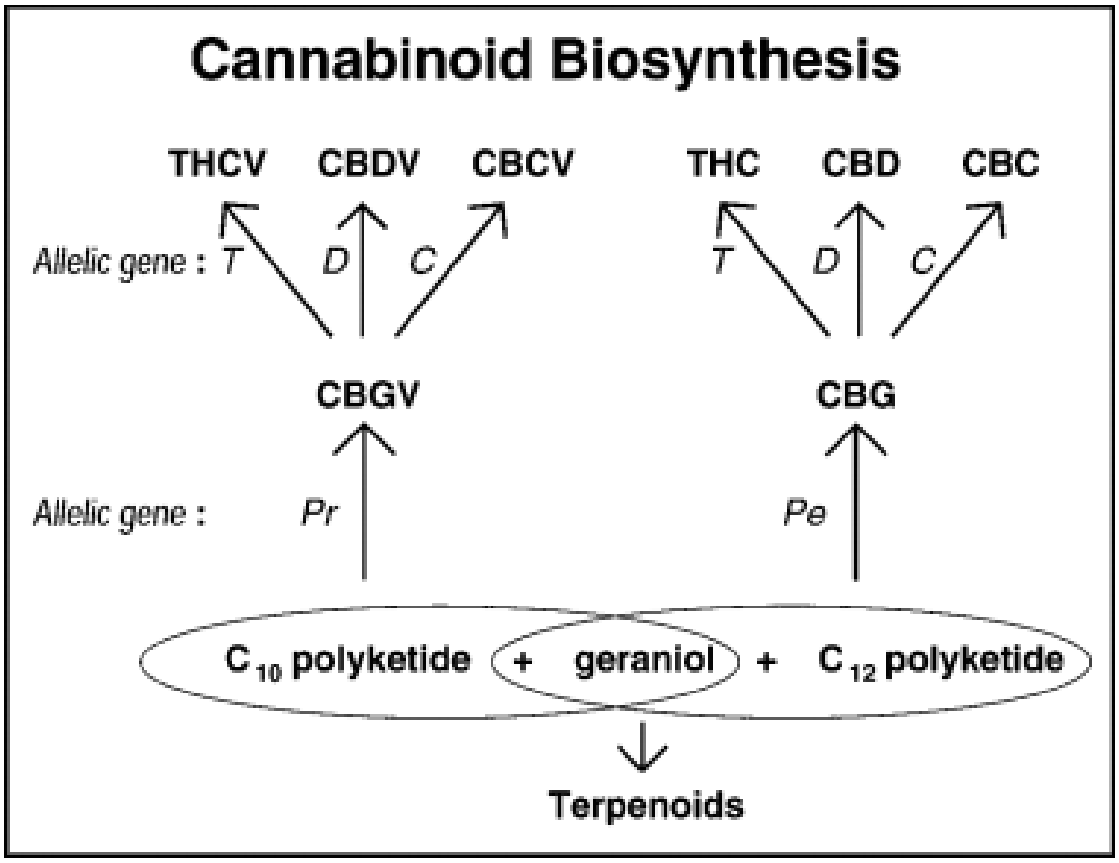


Image 3. Cannabinoid biosynthesis, adapted from (Clarke and Watson, 2007)



Another important factor determining the potency of the plant is whether or not the female plant is pollinated and starts the production of cannabis seeds. Once pollinated, the female plant devotes vast amounts of energy into seed production, while unpollinated plants keep producing cannabinoids in great quantities (Potter, 2013). These unpollinated female plants are commonly referred to as *sinsemilla,* which is Spanish for "without seed". Today, it is this type of T allele dominant sinsemilla cannabis, strains which are high in Δ9-THC and almost devoid of CBD, which dominates the UK black market (Potter et al., 2008). Cannabis resin (also known as hash) which is extracted from the cannabis plant, is usually very high in trichomes which also contributes to its high potency (Pijlman et al., 2005). In the UK however, hash is imported from North Africa where cannabis-farmers select plants which produce the greatest yield rather than potency, resulting in a plant which has roughly equal levels of Δ9-THC and CBD (Potter et al., 2008; Clarke and Watson, 2007). Furthermore, the plants are allowed to become fertilised which also contributes to a lower potency.

Currently in the UK, cannabis is mainly grown indoors under intense lighting conditions (Hardwick and King, 2008). Although this has not been found to affect Δ9-THC potency of the product, it does increase the proportion of floral material resulting in a greater yield (Potter and Duncombe, 2012). As a more potent cannabis product is likely to incur greater profits for the producers, this may explain why there has been a push in the recent decade towards more potent cannabis varieties grown under intense indoor lighting conditions (King et al., 2004; Potter et al., 2008; Hardwick and King, 2008).

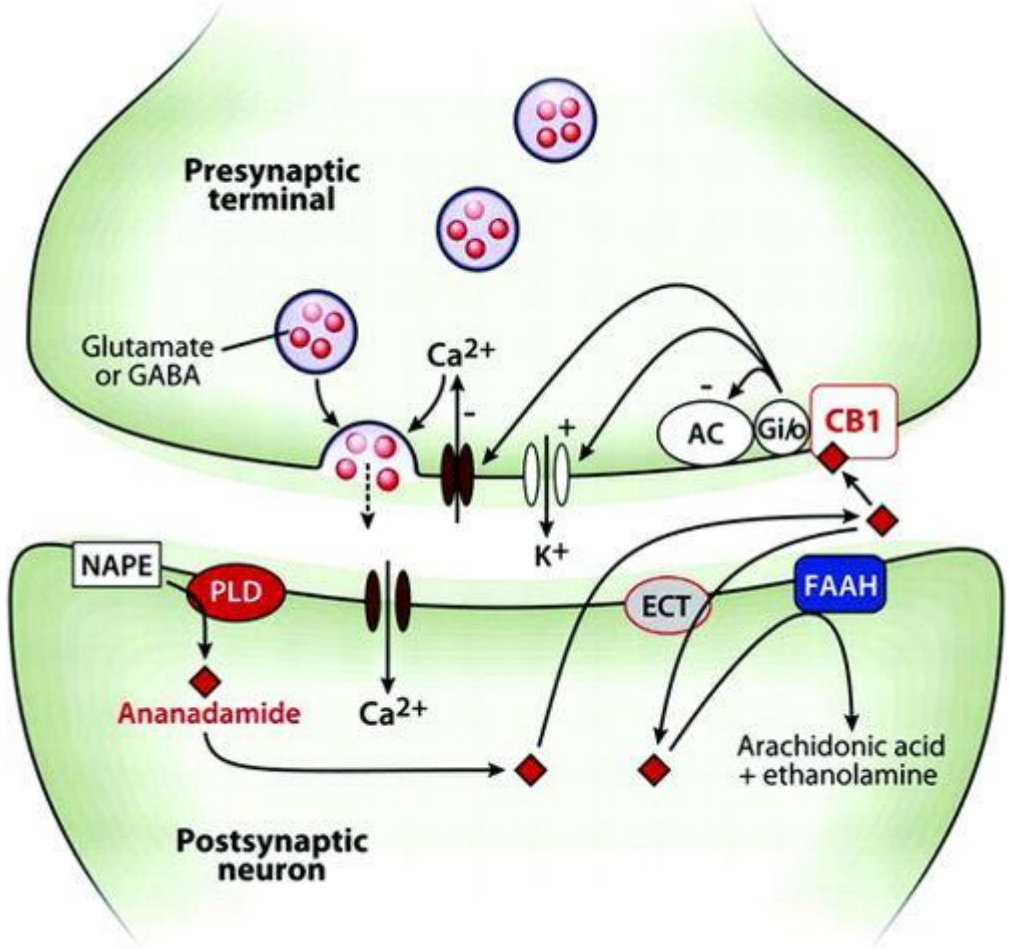
The Endocannabinoid system

The cannabinoids exert their effect on the mind by interacting with the endogenous cannabinoid system (ECS) (Devane et al., 1988) as well as many other pharmacological targets. The endocannabinoid system refers to a set of endogenous ligands, their receptors, and the enzymes that synthesize and degrade them. Twenty years after the discovery of the structure of Δ9-THC (Gaoni et al., 1964) researchers identified a cannabinoid-specific receptor: cannabinoid receptor type-1 (CB1) (Devane et al., 1988), shortly followed by cannabinoid receptor type-2 (CB2) (Munro et al., 1993). The cannabinoid receptors belong to the super-family of G-protein coupled receptors with densities about 10-50 times that of classical neurotransmitters such as opioid or dopamine receptors (Howlett et al., 1990). The CB1 receptor is predominantly found in the central nervous system with the highest concentrations in the neocortex, basal ganglia, hippocampus, cerebellum, and anterior olfactory nucleus (Glass et al., 1997). Moderate concentrations of CB1 are also present in the hypothalamus, basolateral amygdala, and the periaqueductal gray matter in the midbrain. The CB2 receptor was initially thought to be localized only in immune cells in the periphery (Piomelli, 2003), but has more recently also been found in the cerebellum and brain stem (Suárez et al., 2008).

To date, several endogenous cannabinoid receptor ligands have been found; the most well-known are N-arachidonoylethanolamide (Anandamide, AEA) (Devane et al., 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam et al., 1995). These are biosynthesized post-synaptically in an activity-dependant manner (Fernandez-Espejo et al., 2009). CB1 receptors are predominantly pre-synaptic, occurring on the terminals of GABAergic and glutamatergic neurons. The relative dominance of CB1 receptors on GABAergic

neurons vs glutamatergic ones differ depending on brain region. For instance, in the hippocampus, CB1 receptors are found at higher densities on GABAergic neurons compared to glutamatergic (Monory et al., 2006). Activation of CB1 receptors leads to a decrease of pre-synaptic neurotransmitter release. Endocannabinoids regulate GABAergic and glutamatergic over short and long-term durations by adjusting synaptic weight (synaptic plasticity) (Fernandez-Espejo et al., 2009). Clearance of AEA and 2-AG is by a re-uptake mechanism and enzymatic hydrolysis, fatty acid amide hydrolase (FAAH) for AEA and monoacylglyceride lipase (MAGL) for 2-AG (Dinh et al., 2002) (see Image 4).

Image 4. Endocannabinoid signalling, adapted from (Benarroch, 2007)



Endocannabinoid transmission is finely tuned, with precise mechanisms for local synthesis and degradation. Administration of exogenous CB1 agonists is unlikely to capture the subtleties of endocannabinoid signalling. Rather, disruption of endogenous cannabinoid dependent processes is more likely to occur. Also prolonged activation of the CB1 receptor by Δ9-THC can lead to desensitisation and down-regulation of the CB1 receptor (Hirvonen et al., 2012).

Cannabis and cognition

A major concern of cannabis use is its impact on cognitive functioning and learning, especially among younger users. This has biological plausibility as there are high to moderate densities of CB1 receptors in key areas of cognition, such as the hippocampus and frontal cortex (Glass et al., 1997). Many studies have compared long-term heavy cannabis users to non-using healthy controls on various domains of cognitive performance. However, studies have varied greatly in their participant selection criteria and type of task employed (Schoeler and Bhattacharyya, 2013). In a recent longitudinal study, 1,037 participants were followed up from birth up until 38 years of age and were administered standardised neurocognitive test batteries at regular intervals throughout the study. The authors reported a significant decline in IQ-scores of 6-points among cannabis using participants who had been diagnosed with cannabis use disorder (CUD) at 3 testing-points or more, after the age of 18. This performance drop was observed even after 1 year of abstinence, although it was only present for users who had started using cannabis before the age of 18 (Meier et al., 2012). This distinction of effect between different ages of onset highlight the increased risk related to an earlier onset of use on outcome. A recent study which collated data from 3 studies supports this notion as it found adolescent use to be related to poorer educational attainment, psychological functioning and greater likelihood of cannabis dependence (Silins et al., 2014). However, studies such as these are difficult to interpret due to the possibility of confounding variables such as mood disorders, past history of other drug use and academic achievement, as these factors are known to influence the effect sizes (Schoeler and Bhattacharyya, 2013). In fact, a yet to be published longitudinal study in 2,235 adolescents found that the heaviest users showed a 3-point drop in IQ between the ages of 8 and 15 years. However, when

factors such as alcohol, tobacco, other drugs, gender, socioeconomic status, maternal factors and mental health were included in the model, the association between cannabis use and IQ became non-significant (Mokrysz et al., 2014).

Mental health as a confounder was recently studied in a comparison between adolescents with CUD after abstinence, controls with psychiatric disorders, and healthy adolescents. The authors found no difference in academic achievement between adolescents with psychiatric disorders, regardless of cannabis use. Furthermore, they noted that abstinent adolescents did not significantly differ compared to the other groups (Hooper et al., 2014). Seemingly, heavy cannabis users regain their cognitive abilities following extended periods of abstinence, which suggests that the detriments may be explained by withdrawal symptoms or recovering from the last use occasion. (Pope et al., 2001; Fried et al., 2005; Hanson et al., 2010; Tait et al., 2011). This has also recently been confirmed in a meta-analysis where it was concluded that neurocognitive impairments do not persist following 25 days of abstinence (Schreiner and Dunn, 2012). This field of study is further confounded by the fact that studies to date have been unable to control for cannabis type, where some may contain higher or lower levels of Δ9-THC or a presence or absence of CBD.

Studies of the acute effects of cannabis and Δ9-THC on cognitive performance started in the early 1970s, when converging lines of evidence highlighted impairments to performance in a dose response manner (Tinklenberg et al., 1970; Miller and Cornett, 1978). Δ9-THC impacts most areas of cognition negatively when given in high enough doses, although poorer performance is less marked in certain areas of executive functioning (Pope et al., 1995) such as verbal fluency (Morrison et al., 2009) and risk-taking (Ramaekers et al., 2006a). The domains of cognition which are most robustly impacted by Δ9-THC are those of immediate and delayed recall (verbal and digit) (Ranganathan and D'Souza, 2006; Schoeler and Bhattacharyya, 2013), whereas learning (D'Souza et al., 2004; Morrison et al., 2009; D'Souza, Braley, et al., 2008) and recognition recall are not affected (D'Souza et al., 2004; D'Souza, Ranganathan, et al., 2008; Liem-Moolenaar et al., 2010; Kleinloog et al., 2012). Also, heavy users are not as negatively affected by cannabis as occasional users (Ramaekers et al., 2009; Hart et al., 2001, 2002). This was highlighted in a study administering large intravenous doses to frequent and occasional users (D'Souza, Ranganathan, et al., 2008), which showed

heavy users performing worse on both immediate and delayed recall compared to occasional users. However, for each subsequent increased dose of Δ9-THC, the frequent users outperformed the controls and the memory impairment was significantly less pronounced in the frequent users. The poor baseline performance which the heavy users showed might be due to withdrawal experienced during the drug-free baseline condition. This would then account for the stable or improved performance among frequent users following THC administration, while occasional users would display impairment.

A few studies have explored the potential inhibitory effects of the commonly prescribed antipsychotic medications Haloperidol and Olanzapine on Δ9-THC effects in healthy volunteers. These studies have found that both antipsychotics and Δ9-THC impair immediate and delayed recall on their own to a comparable degree (D'Souza, Braley, et al., 2008; Kleinloog et al., 2012; Liem-Moolenaar et al., 2010). However, when administered together, antipsychotics further exacerbate the cognitively impairing effects of Δ9-THC. These results suggest that the cognitively impairing effects of cannabis and Δ9-THC does not relate directly to the dopaminergic system, which these drugs target.

Cannabidiol has been shown in animal models of cognitive impairment to improve cognition (Barichello et al., 2012; Avraham et al., 2011; Magen et al., 2010; Fagherazzi et al., 2012), as well as protecting against the memory impairing effects of Δ9-THC (Fadda et al., 2004). In a naturalistic study, Morgan and colleagues took samples of test subjects' own cannabis to analyse for cannabinoid content and had them perform cognitive tasks once while drug free and once while intoxicated. They found that participants who smoked cannabis higher in CBD did not show any impairments in immediate or delayed recall during intoxication, while the participants who smoked cannabis with only Δ9-THC were significantly impaired (Morgan, Schafer, et al., 2010). The same group later studied daily and recreational cannabis users and collected hair samples which were analysed for Δ9-THC and CBD content. When comparing the two groups, the authors found a significant improvement in recognition memory in the group that had CBD present in hair samples (Morgan et al., 2012). Taken together, these studies highlight the cognitively protective properties of CBD against Δ9-THC induced impairments. They also suggest that manipulation of the endocannabinoid

system (using other cannabinoids such as CBD or Δ9-THCV) may serve as a better target against the memory impairing effects of cannabis rather than the dopaminergic system. However, no study to date has studied the protective effects of CBD on Δ9-THC in humans using carefully controlled doses of the two compounds.

Cannabis and psychosis

> "...ma-fen (fruit of the cannabis plant), if taken in excess will produce visions of devils ... over a long term, it makes one communicate with spirits and lightens one's body..."
>
> - Pen-ts'ao ching, Chinese pharmacopeia 2700 BC

Although there had been many suspicions of a link between cannabis use and psychotic illness, the first real evidence came from a large cohort study from Sweden in 1987. The study followed roughly 50,000 military conscripts for 15 years and showed that people who had reported cannabis use at conscription had an increased likelihood of being diagnosed with schizophrenia at follow-up (Andréasson et al., 1987). Furthermore, they found that the heaviest users had a 6-fold increased risk compared to non-users. Although this study received much attention, it was not until the early and mid 2000s that further research began to emerge. These studies lend further support to the association between the use of cannabis and psychotic illness, with an earlier onset and more frequent use being related to higher risk (Arseneault et al., 2002; Fergusson et al., 2005; van Os, 2002). In 2007, a meta-analysis put together the results of the best studies to date and found that having ever used cannabis increased the risk of psychotic outcome by 40%, and for daily users the risk was doubled (Moore et al., 2007). However, a few newer studies have highlighted nuances to this relationship. A recent case-control study found that the use of stronger cannabis incurred an even greater risk of psychosis while use of weaker cannabis was not related to an increased risk (Di Forti et al., 2015). Furthermore, a gene which is involved in the dopaminergic signalling cascade in the striatum by coding for a certain protein kinase, AKT1, has surfaced as a potential mediating factor between cannabis and psychosis. A recent case-control study found that only carriers of the C/C variant of the AKT1 gene had an elevated risk of psychosis when they used cannabis every day

(Di Forti et al., 2012). AKT1 has also been found to modulate the cognitive effects of cannabis in a population of psychotic patients (van Winkel et al., 2011). Another gene which holds theoretical plausibility is the gene which codes for the CB1 receptor, CNR1. Studies to date have yielded mixed findings with regards to variations of the CNR1 gene and psychosis (Chavarría-Siles et al., 2008; Martínez-Gras et al., 2006; Leroy et al., 2001; Seifert et al., 2007; Tsai et al., 2000; Zammit et al., 2007; Ujike et al., 2002). The largest of these studies failed to find an association between the CNR1 gene and psychosis, nor an interaction between the CNR1 gene and cannabis use (Zammit et al., 2007).

The issue of whether cannabis is causality related to psychotic illness has still not been adequately addressed. A longitudinal study showed that although cannabis use at age 16 predicted psychosis at age 19, psychotic symptoms at age 13 and 16 predicted cannabis use at age 16 and 19 respectively (Griffith-Lendering et al., 2013). Studies such as this and others like it (Cassidy et al., 2011; Ferdinand et al., 2005) suggest that the relationship might be bi-directional, highlighting the alternative explanation of psychotic symptoms or vulnerability might make cannabis use more likely, and vice versa. Furthermore, there may be other mediating factors which both increase risk for psychosis as well as increase likelihood of heavy cannabis use. For instance, familial risk (having relatives with psychotic illness) has been found to explain the risk between cannabis use and psychotic illness (Proal et al., 2014), although this may be explained as cannabis triggering an underlying vulnerability (McGuire et al., 1995). Twin studies have also highlighted that heavy cannabis use has a strong genetic component (Kendler and Prescott, 1998; Kendler et al., 2002). Although environmental factors play a clear role in whether or not someone tries cannabis, the heritability of heavy cannabis use, abuse and dependence is significantly higher and ranges between 45-78% across studies (Lynskey et al., 2002; van den Bree et al., 1998; Rhee et al., 2003; Kendler and Prescott, 1998; Kendler et al., 2000). Examining the literature more closely it becomes evident that there is greater heritability for heavy cannabis use compared to dependence (79% vs 62% (Kendler and Prescott, 1998), 84% vs 58% (Kendler et al., 2000),64% vs 45% (Lynskey et al., 2002)), which suggests that environmental factors play a greater role in the occurrence of dependence. Furthermore, there is a significant coherence between subjective effects where roughly a quarter of the variance for both

negative and positive cannabis effects were determined by genetic factors (Lyons et al., 1997).

A significant confound for the association between cannabis use and psychosis is use of tobacco, as cannabis is commonly smoked together with tobacco. Tobacco use is highly prevalent among patients with psychosis and a meta-analysis found a strong association between psychosis and tobacco use (Myles, H. D. Newall, et al., 2012), although a separate meta-analysis found no association with age at onset of psychosis (Myles, H. Newall, et al., 2012). However, a recent study found that age at first tobacco use was related to several measures of psychotic like experiences and still related to hallucinations when all participants with any cannabis experience were excluded (McGrath et al., 2015). In a cross-sectional survey study, use of tobacco and cannabis were equally strongly associated with psychotic like experiences, although the association between cannabis and psychotic like experiences became non-significant when controlling for tobacco (van Gastel et al., 2013). Similar results were found in a longitudinal study of adolescents, where both use of cannabis and tobacco at age 16 were equally related to psychotic like experiences at age 18 (Gage et al., 2014). Additional longitudinal studies with more precise measures of cannabis and tobacco exposures are needed to disentangle these associations.

From the studies presented above it is clear that the association between cannabis use and psychosis is highly complex and far from straight forward. However, it is widely accepted today that cannabis use is a component cause, which implies that it is not a necessary or sufficient cause of psychotic illness, but potentially a mediating or catalytic factor (van Winkel and Kuepper, 2014; Murray et al., 2007).

The acute psychotic-like effects of cannabis are far less controversial or contentious. In as early as the mid-1800s, the French psychiatrist Jacques-Joseph Moreau extensively reported the effects of eating large quantities of hash and encouraged his students to do the same (Moreau J, 1845). He considered psychoactive substances to be both the cure and cause of mental illness, and famously said: “I saw in hashish, more specifically in its effects on mental abilities, a powerful and unique method to investigate the genesis of mental illness”. Although the psychotogenic effects of cannabis had been reported in the literature between the 1950s to 1980s in studies and observations

(Ames, 1958; Talbott, 1969; Chopra, 1974; Isbell et al., 1967; Melges, 1976), these reports did not make use of standardised clinical scales to assess the severity and intensity of these symptoms. It would take until 2004 when D'Souza and colleagues studied the effects of Δ9-THC in 22 healthy volunteers using the Positive and Negative Syndrome Scale (PANSS). They showed that Δ9-THC, in a dose dependent manner increased positive, negative and general psychotic symptoms (D'Souza et al., 2004) as measured by the PANSS. They later went on to show that Δ9-THC would also induce psychotic symptoms or exacerbate pre-existing symptoms in patients with schizophrenia who were symptomatically stable on medication (D'Souza et al., 2005). These findings were later replicated by Morrison and colleagues where Δ9-THC induced psychotic symptoms on not only the PANSS, but also a self-rated scale known as the Community Assessment of Psychic Experiences (CAPE) (Morrison et al., 2009). Interestingly, although these studied administered doses considered much higher than what most recreational users would take (Englund et al., 2012), only 40-50% of healthy participants experienced psychotic symptoms, and inter-individual variation was high (Morrison et al., 2009; D'Souza et al., 2004).

A compound which is capable of inducing psychotic symptoms in a subset of healthy individuals lends itself as being a tool to study the mechanics and possible treatment of psychosis. This has been particularly true for amphetamine or cocaine induced psychosis, where dopamine blocking drugs such as haloperidol are effective at alleviating psychotic symptoms (Awouters and Lewi, 2007). In line with this thought of reasoning, drugs which block the psychotogenic effects of Δ9-THC may also be effective in treating psychotic illnesses such as schizophrenia. Similarly, drugs which are effective at treating symptoms of psychosis may also be effective at reducing the psychotogenic effects of cannabis. A few studies to date have explored the effects of co-administering anti-psychotic drugs with Δ9-THC to healthy volunteers. The study with the most reliable administration route, intravenous (IV) injection of Δ9-THC, showed that pre-treatment with haloperidol did not significantly reduce the psychotogenic effects of Δ9-THC and further worsened the cognitive effects of the injection (D'Souza, Braley, et al., 2008). Two studies have looked at the effects of haloperidol and olanzapine on inhaled Δ9-THC in healthy volunteers and found significant yet weak reductions in psychotic symptoms (Kleinloog et al., 2012; Liem-

Moolenaar et al., 2010). These findings are in line with most studies in healthy volunteers showing no significant increase of dopamine in the striatum following administration of Δ9-THC (Barkus et al., 2011; Stokes et al., 2009; Bloomfield et al., 2013; Bossong et al., 2009). However, a recent study did show that dopamine was significantly increased by Δ9-THC, but only among patients with schizophrenia and their relatives (Kuepper et al., 2013). A recent study which combined data from two separate PET studies found that Δ9-THC did significantly increase striatal dopamine levels in healthy volunteers, although not to a level which would be expected in psychosis (Bossong et al., 2015). Furthermore, a PET study in healthy volunteers did find significant dopamine release following THC in the right middle frontal gyrus, left superior frontal gyrus and left superior temporal gyrus; although none of these increases were related to psychotic symptoms (Stokes et al., 2010). Seemingly, it appears that the dopamine system might be less involved with Δ9-THC induced psychotic symptoms, apart from those who have a family history of psychotic illness.

Cannabidiol has long been known to offset many of the effects of Δ9-THC. The very first report came in the mid-70s when Karniol and colleagues administered oral tablets of both Δ9-THC and CBD to healthy volunteers. In this study co-administration of CBD significantly reduced the heart-rate increase, time-distortion and psychological effects of Δ9-THC (Karniol et al., 1974). A few years later, the same group showed that CBD also reduced the anxiogenic effects of an oral dose of Δ9-THC (Zuardi et al., 1982). More recently, Morgan and colleagues investigated the impact that CBD had on cannabis used recreationally among healthy cannabis users. They analysed participants hair samples for cannabinoids and found that individuals who were positive for Δ9-THC-only had experienced significantly more psychotic like experiences compared to the participants who were positive for both Δ9-THC and CBD (Morgan and Curran, 2008).

Similar results were found in a large Dutch population based study, where participants were asked in an online survey which type of cannabis they preferred smoking. Since the sale of cannabis is tolerated in the Netherlands, this allows government bodies to carry out annual analyses on the cannabinoid content of the various types sold. In the survey, the authors found that users who preferred cannabis types high in CBD had fewer lifetime psychotic experiences (Schubart et al., 2011). Lastly, and possibly the

clearest examples of CBD's ability to inhibit the psychotogenic effects of Δ9-THC, was a small pilot-study administering Δ9-THC and CBD to 6 healthy volunteers intravenously. 1.25mg IV Δ9-THC significantly increased psychotic symptoms, while this was significantly reduced by co-administration of 5mg IV CBD (Morrison et al., 2010).

Intravenous studies

In order to study specific drug mechanisms and interactions, it is vital to minimise any source of variability in bioavailability of the compounds being tested. Cannabis is most commonly smoked as cigarettes or ingested orally. Naturalistic studies exploring the effects of recreational cannabis use on various outcomes benefit from allowing the participant to smoke or ingest the drug in a manner as similar to real life as possible (Morgan, Schafer, et al., 2010). One drawback however is that there are significant inter-individual differences in drug absorption from both oral and smoked routes of administration. For smoked cannabis, factors such as duration of smoking, puff duration, volume inhaled, smoking skill, lung capacity and loss of side-stream smoke may affect bloodstream cannabinoid concentration (Lindgren et al., 1981; Perez-Reyes et al., 1982). Orally consumed cannabinoids (either via capsules or food items) suffer from poor and irregular absorption (Grotenhermen, 2003), and are pharmacologically the least reliable. It is estimated that about 50% of the cannabinoids are lost due to stomach acids (Perez-Reyes et al., 1973). Intravenous administration on the other hand provides the most reliable delivery of synthetically prepared cannabinoids, with low inter-individual variability in drug plasma concentrations (Ohlsson et al., 1981). The plasma profile following an intravenous dose approximates that from the inhalational route (Ohlsson et al., 1981) where concentrations fall rapidly due to redistribution. Further reductions, attributable to drug metabolism, progress at a slower rate. Table 1 provides an overview of IV cannabinoid studies from the early 1970s to the present day.

Table 1. List of intravenous cannabinoid studies, adapted from (Englund et al., 2012)

Table 1. List of Intravenous Cannabinoid Studies with Cannabinoid Used, Dose, Duration of Infusion, and Outcome

Year	Author	Participants	Cannabinoid given	Dose	Duration of infusion	Psychotic symptoms	Cognitive impairment	Observations
1970	Lemberger *et al.* [39]	3 healthy volunteers	Δ9-THC	0.5mg	Not reported	Not reported	Not reported	N/A
1972	Lemberger *et al.* [40]	12 long-term users	Δ9-THC	0.5mg	Not reported	Not reported	Not reported	N/A
1972	Lemberger *et al.* [41]	3 infrequent users	11-OH-Δ9-THC	1mg	Not reported	Not reported	Not reported	Participants reported greater "high" than they had experienced from smoking
1972	Hollister and Gillespie [42]	4 healthy volunteers	Δ9-THC	1-6mg	Not reported	Visual perception changes, mental confusion, "in-and-out" feeling.	Poor memory and difficulty concentrating (3mg and over)	Δ8-THC is slightly less potent than Δ9-THC
		3 healthy volunteers	Δ8-THC	1-9mg	Not reported	Similar to Δ9-THC	Similar to Δ9-THC	
1973	Perez-Reyes *et* . [43]	6 healthy volunteers	Δ9-THC	3.10±0.9mg	15-25 min	Not reported	Not reported	Δ9-THC produced a
		6 healthy volunteers	11-OH-Δ9-THC	2.27±0.8mg	15-25 min	Not reported	Not reported	longer lasting and more intense 'high' than its metabolite
1973	Perez-Reyes *et* . [44]	21 healthy volunteers	Δ9-THC	~ 4mg	15-25 min	Not reported	Not reported	Participants reported never have been so "high" from smoking cannabis after IV Δ9-THC
		6 healthy volunteers	CBN	~ 20mg	15-25 min	Not reported	Not reported	CBN produced a mild "high" at the higest dose.
		6 healthy volunteers	CBD	~ 20mg	15-25 min	Not reported	Not reported	CBD did not produce any psychological effect at any dose
1973	Hollister [45]	4 healthy volunteers	CBD	5-30mg	Not reported	Not reported	Not reported	CBD did not produce any psychological effect at any dose
1974	Perez-Reyes *et al.* [46]	30 frequent and infrequent users	Δ9-THC	53-68 μg/kg (3.71-4.76mg for 70kg)	15-25 min	Not reported	Not reported	N/A
1974	Perez-Reyes and Wingfield [47]	One epileptic patient	CBD	40mg	~ 16 min	Not reported	Not reported	N/A

(Table 1) Contd....

Year	Author	Participants	Cannabinoid given	Dose	Duration of infusion	Psychotic symptoms	Cognitive impairment	Observations
1974	Hollister [48]	6 healthy volunteers	Δ9-THCV	7mg	Not reported	Not reported	Not reported	Mild to moderate side effects, similar to Δ9-THC reported
1977	Raft *et* . [49]	10 healthy volunteers	Δ9-THC	0.022-0.044 mg/kg (1.54-3.08mg for 70kg)	Not reported	Increased anxiety	Not reported	N/A
1980	Hunt and Jones [50]	6 healthy volunteers	Δ9-THC	2mg	15 min	Not reported	Not reported	N/A
1980	Ohlsson *et* . [38]	11 healthy volunteers	Δ9-THC	5mg	2 min	Not reported	Not reported	N/A
1981	Lindgren *et* . [34]	18 light and heavy users	Δ9-THC	5mg	2 min	Not reported	Not reported	Light users were more intoxicated by IV Δ9-THC than heavy users
1983	Wall *et* . [51]	12 healthy volunteers	Δ9-THC	2.2-4mg	15-25 min	Not reported	Not reported	N/A
1991	Volkow *et* . [52]	8 occasional users	Δ9-THC	2mg	Not reported	Anxiety and paraoia in 2 participants	Not reported	3 out of 8 rated the experience as unpleasant
2004	Naef *et* . [53]	8 cannabis naïve subjects	Δ9-THC	0.053mg/KG (3.71mg for 70kg)	2 min	Anxiety, hallucinations, perceptual change, strange ideas/mood	Not reported	No observed analgesic effect of Δ9-THC
2004	D'Souza *et* . [54]	22 infrequent users	Δ9-THC	2.5 and 5mg	2 min	Paranoia, grandiose delusions, conceptual disorganisation, illusions, depersonalisation, slowing of time, blunted affect, emotional withdrawal, lack of spontaneity	Immediate, delayed recall and learning. Working memory for 'easy' task.	Verbal fluency and working memory for 'hard' task remained intact.
2005	D'Souza *et* . [55]	13 stable (medicated) schizophrenic patients	Δ9-THC	2.5 and 5mg	2 min	Worsening in positive, negative, and general psychotic symptoms (PANSS). Increased perceptual alterations (CADSS).	Immediate/delayed recall, learning and vigilance	Worsening of anti-psychotic side effects
2008	D'Souza *et* . [56]	30 frequent users	Δ9-THC	2.5 and 5mg	2 min	Perceptual alterations and psychotomimetic effects.	Immediate, delayed recall and learning.	Although Δ9-THC produced psychotic symptoms and cognitive impairments, this was significantly less compared to controls

(Table 1) Contd....

Year	Author	Participants	Cannabinoid given	Dose	Duration of infusion	Psychotic symptoms	Cognitive impairment	Observations
2008	D'Souza *et* . [57]	28 frequent and infrequent users	Δ9-THC (Haoperidol pretreatment)	0.0286mg/kg (2mg for 70kg)	20 min	Perceptual alterations and psychotomimetic effects.	Immediate, delayed recall and learning.	Pretreatment with Haloperidol worsened cognitive performance under Δ9-THC condition
2009	Zuurman *et* . [58]	21 infrequent users	Org 28611 (potent CB1 agonist)	0.3-10µg/kg (0.021-0.7mg for 70kg)	1 or 25 min (bolus or slow infusion)	Delusional perception, derealisation, confusional state, hallucinations	Attention	No adverese effects were experienced from bolus dose of 3µg/kg and less
2009	Morrison *et* . [59]	22 healthy volunteers	Δ9-THC	2.5mg	5 min	Positive symptoms (PANSS, CAPE), anxiety	Working memory, executive function	Psychotic symptoms were not related to levels of cognitive impairment or anxiety
2010	Bhattacharyya *et* . [60]	6 healthy volunteers	Δ9-THC, with CBD or placebo pretreatment	1.25mg (Δ9-THC) 5mg (CBD)	5 min 5 min	Positive symptoms (PANSS)	Not reported	Pretreatment with CBD protected against psychotomimetic effects of Δ9-THC
2010	Barkus *et* . [61]	10 healthy volunteers	Δ9-THC	2.5mg	5 min	Positive and general symptoms (PANSS)	Not reported	No significant dopamine release in the striatum following Δ9-THC
2010	Stone *et* . [62]	16 healthy volunteers	Δ9-THC	1.25mg	Not reported	Not reported	Subjective impairments to attention and concentration	Impaired time perception and estimation
2010	Morrison *et* . [63]	16 healthy volunteers	Δ9-THC	1.25mg	5 min	Positive, negative and general symptoms (PANSS)	Reduced accuracy on the hardest working memory task	Neural synchronicity (Theta-coherence) associated with psychotic symptoms
2011	Stone *et* . [64]	16 healthy volunteers	Δ9-THC	1.25mg	5 min	Paranoid ideations, anxiety, salience, identity disturbance, perceptual abnormalities	Not reported	Neural synchronicity (Inter-trial-coherence) associated with salience and identity disturbance
2011	Morrison *et* [65]	22 healthy volunteers	Δ9-THC	2.5mg	5 min	Negatice psychotic symptoms (PANSS, CAPE-state)	Not reported	Negative symptoms were not associated with sedation

There is wide variation and individual reactions to IV Δ9-THC. This is illustrated by IV studies administering high doses of Δ9-THC to healthy volunteers, and observing psychotic symptoms, anxiety, and dysphoria. Volkow and colleagues administered a 2mg IV dose of Δ9-THC to 8 healthy volunteers, of whom two became anxious and one became paranoid (Volkow et al., 1991). In a study Morrison and colleagues, 50% of participants experienced increased positive psychotic symptoms following a 2.5mg IV

Δ9-THC dose (Morrison et al., 2009). D'Souza and colleagues administered 2.5mg and 5mg IV Δ9-THC to both healthy volunteers and clinically stable schizophrenic patients. They observed that 35% of the controls and 80% of the patients displayed psychotic symptoms following 2.5mg, while 50% of controls and 75% of patients did so after 5mg (D'Souza et al., 2005). Jointly, these observations suggest that about 35-50% of the psychiatrically healthy general population are susceptible to psychotomimetic effects and anxiety experienced from high doses of Δ9-THC. Conversely, this means about half the general population are in some way resilient towards these effects. One possible explanation to this may be that these individuals have a naturally higher level of endocannabinoid activity. It has been previously shown that schizophrenic patients which higher levels of CSF anandamide are less symptomatic (Giuffrida et al., 2004) and prodromal patients with high anandamide transition into psychosis later (Koethe et al., 2009).

Although the intravenous route of administration has clear benefits in terms of elucidating precise pharmacological effects and interactions, it does suffer limitations in terms of how generalisable it is when drawing conclusions about cannabis use. Cannabis is commonly smoked with tobacco and titrated to achieve desired effects (T. P. Freeman et al., 2014), although a recent study has found that this may be more difficult with higher potency products (van der Pol et al., 2014). In studies which administer an intravenous bolus the participant is unable to titrate the dose of the cannabinoids resulting in a dose which is not compatible to the one he/she would use recreationally. Furthermore, studying the effects of cannabis in a laboratory environment may fail to provide ecological validity as the setting may feel unnatural or intimidating to the participant (Morgan, Schafer, et al., 2010). Lastly, cannabis which is ingested orally has a vastly different metabolism which results in increased levels of psychoactive metabolites (eg. 11-OH-Δ9-THC and 7-OH-CBD) compared to inhaled or IV, which may result in a different psychopharmacological effect (Ohlsson et al., 1981).

Aims

In this thesis I aim to explore the psychological, cognitive, and electrophysiological effects of different cannabinoids in healthy volunteers, making use of the intravenous administration route to ensure minimal variation in bioavailability of Δ9-THC. I will do

this across two separate studies. The first study will pre-treat participants with either CBD or placebo before IV administration of 1.5mg Δ9-THC. The psychological and cognitive effects of this study will be presented separately to the electrophysiological, in different chapters. The second study will pre-treat volunteers with Δ9-THCV or placebo before administration of 1mg IV Δ9-THC. I hypothesise that CBD and Δ9-THCV will significantly reduce the psychotomimetic and cognitively impairing effects of IV Δ9-THC, and that 1mg Δ9-THC will have less negative effects compared to 1.5mg. Furthermore, I predict that CBD will significantly reduce Δ9-THC-induced electrophysiological abnormalities.

Cognitive and psychological effects of Δ9-THC and CBD

Pre-article introduction

CBD was first isolated in the 1930s and its structure was elucidated in 1963 by Mechoulam and colleagues (Mechoulam and Shvo, 1963). In a study comparing the effects of smoked cannabis or pure Δ9-THC, the authors noted there being a qualitative difference between the two smoked preparations (Galanter, 1973). This was later confirmed in both animal and human studies, which seem to suggest that other components of the cannabis plant interact with Δ9-THC and change its effects (Carlini et al., 1974). The first study to compare the effects of pure CBD and Δ9-THC in humans took place in 1974, where oral doses of Δ9-THC (30mg) were combined with increasing doses of CBD (15mg, 30mg, and 60mg) (Karniol et al., 1974). They found that CBD was able to reduce the heart rate increase and time-underestimation produced by Δ9-THC, while also producing fewer unpleasant experiences. In a subsequent study 1mg/kg CBD significantly reduced the anxiety produced by 0.5mg/kg Δ9-THC when given orally, further suggesting CBD acts in an inhibitory fashion against Δ9-THC.

As will be mentioned in the article below, more recent naturalistic studies have found that cannabis products which contain higher levels of CBD are less psychotogenic and cognitively impairing (Morgan and Curran, 2008; Schubart et al., 2011). Epidemiological studies have also suggested that cannabis with higher CBD content poses less risk of psychosis (Di Forti et al., 2009). As previously mentioned, orally or inhaled Δ9-THC suffers from unpredictable absorption and irregular inter-individual bioavailability (Grotenhermen, 2003). The aim of the below study is to explore the protective effects of CBD on Δ9-THC induced paranoia, psychosis and memory impairment, making use of IV Δ9-THC administration to reduce inter-individual bioavailability.

The initial plan of the below study was to use an intravenous administration for both Δ9-THC and CBD, although the license to import IV CBD had expired and we were forced to use oral CBD instead. Oral CBD goes through first pass metabolism and may result in significantly higher levels of its metabolites (Harvey and Mechoulam, 1990),

which may serve as a confound if they are pharmacologically active or interfere with the pharmacology of CBD and Δ9-THC. Furthermore, the method of using two modes of administration (oral and intravenous) lacks ecological validity, as a user would be exposed to both cannabinoids simultaneously when either inhaling or ingesting cannabis.

The choice of the dose for oral CBD (600mg) was based on previously shown anxiolytic (Bergamaschi et al., 2011) and anti-psychotic effect (Leweke et al., 2012), as well as preliminary pharmacokinetic data from a previous study (Bhattacharyya et al., 2010). The dose of IV Δ9-THC (1.5mg) was based on previous studies from our lab (Morrison et al., 2009, 2011; Barkus et al., 2011) where both the 1.25mg and 2.5mg doses significantly impaired cognition and induced psychotic symptoms, the latter dose being found to be subjectively very unpleasant for study participants.

Cannabidiol inhibits Δ9-THC-elicited paranoid symptoms and hippocampal-dependent memory impairment

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Abstract

Community-based studies suggest that cannabis products that are high in Δ^9-tetrahydrocannabinol (Δ9-THC) but low in cannabidiol (CBD) are particularly hazardous for mental health. Laboratory-based studies are ideal for clarifying this issue because Δ9-THC and CBD can be administered in pure form, under controlled conditions. In a between-subjects design, we tested the hypothesis that pre-treatment with CBD inhibited Δ9-THC-elicited psychosis and cognitive impairment. Healthy participants were randomised to receive oral CBD 600mg (*n*=22) or placebo (*n*=26), 210 min ahead of intravenous (IV) Δ9-THC (1.5 mg). Post-Δ9-THC, there were lower PANSS positive scores in the CBD group, but this did not reach statistical significance. However, clinically significant positive psychotic symptoms (defined a priori as increases ≥3 points) were less likely in the CBD group compared with the placebo group, odds ratio (OR)=0.22 (χ^2=4.74, p<0.05). In agreement, post-Δ9-THC paranoia, as rated with the State Social Paranoia Scale (SSPS), was less in the CBD group compared with the placebo group (t=2.28, p<0.05). Episodic memory, indexed by scores on the Hopkins Verbal Learning Task-revised (HVLT-R), was poorer, relative to baseline, in the placebo

pre-treated group (-10.6±18.9%) compared with the CBD group (-0.4%±9.7 %) (t=2.39, p<0.05). These findings support the idea that high-Δ9-THC/low-CBD cannabis products are associated with increased risks for mental health.

Introduction

The cannabis plant contains over 60 different cannabinoid molecules(Izzo et al., 2009), but two in particular have relevance for psychiatry. Δ^9-tetrahydrocannabinol can induce acute psychotic symptoms, in medicated schizophrenic patients and in healthy controls, whereas cannabidiol (CBD) is showing promise as a possible anti-psychotic (D'Souza et al., 2009; Leweke et al., 2000; Zuardi et al., 2006).

The balance of these two molecules in 'street cannabis' appears to have changed over the last decade. For example, in the UK and Holland, cannabis products traditionally contained about 4% Δ9-THC and 4% CBD, as compared with 16–22% Δ9-THC and <0.1% CBD content in modern 'high-potency' products (Sinsemilla or 'skunk') (Slade et al., 2012). There is accruing evidence that sinsemilla carries a greater risk to mental health (Di Forti et al., 2009; Morgan and Curran, 2008; Schubart et al., 2011).

In a highly original design, Morgan and Curran measured trace cannabinoid levels in hair samples from regular cannabis users as well as psychosis proneness as rated by the OLIFE (Oxford Liverpool Inventory of Life Experiences) instrument. Regular users who were grouped as Δ9-THC-positive/CBD-negative scored higher on scores of unusual experiences than regular users who were positive for both cannabinoids (Morgan and Curran, 2008). In an epidemiological study in South London, Di Forti and colleagues compared patterns of drug use in people presenting with a first episode of psychosis with healthy controls. Patients were approximately seven times more likely than controls to be users of sinsemilla (Di Forti et al., 2009).

In Holland, the most popular types of cannabis sold are measured annually for Δ9-THC and CBD content. Schubart and colleagues combined this information with data on cannabis use from approximately 1900 people, and found that the Δ9-THC/CBD ratio was related to subclinical psychotic experiences as rated by the CAPE scale (Community Assessment of Psychic Experiences). Subjects who used products with a high Δ9-THC/CBD ratio reported significantly higher CAPE-total scores than those using

products with a low Δ9-THC/CBD ratio. In heavy users, higher CBD content was associated with lower scores on the CAPE-positive symptoms dimension (Schubart et al., 2011).

In laboratory-based experimental studies, the acute effects of specific cannabinoid molecules can be measured under tightly controlled conditions. For example, in the early 1980s, Zuardi and colleagues demonstrated that CBD (1 mg/kg) inhibited the anxiety provoked by Δ9-THC (0.5 mg/kg) (Zuardi et al., 1982). More recently, in a neuroimaging study of 15 healthy volunteers, task-specific blood-oxygen-level-dependent (BOLD) responses were measured following the administration of oral Δ9-THC (10 mg), CBD (600 mg) or placebo. Relative to placebo, Δ9-THC and CBD evoked diametrically opposite task-specific BOLD responses in the hippocampus, the amygdala and the occipital cortex (Bhattacharyya et al., 2010), the right superior temporal gyrus (Winton-Brown et al., 2011) and the pre-frontal cortex and caudate nucleus (Bhattacharyya et al., 2012).

Previously we reported preliminary findings that pre-treatment with intravenous (IV) CBD (5 mg) inhibited IV Δ9-THC (1.25 mg) evoked positive psychotic symptoms, as measured by the Positive & Negative Syndrome Scale (PANSS), although the small sample size (crossover, *n*=6) prevents definitive conclusions (Morrison et al., 2010). Here we report the first findings from a larger study (between groups, *n*=48) in which IV Δ9-THC (1.5 mg) followed pre-treatment with either oral CBD (600 mg) or placebo. We hypothesised that, following IV Δ9-THC, the group who had been pre-treated with CBD would show less positive symptoms and less cognitive impairment than the group that had been pre-treated with placebo.

Methods

The study was approved by the Joint Institute of Psychiatry and Maudsley Hospital Ethics Committee. All subjects provided written informed consent. Safety protocols have previously been described (Morrison et al., 2009).

Design

In a 2×3 mixed design, participants were randomly allocated in a counterbalanced fashion to placebo or CBD groups. Placebo/CBD capsules were administered under double-blind conditions. Each participant was assessed at three separate time-points: (1) baseline; (2) post-capsule; and (3) post-Δ9-THC. (Baseline data were collected on a separate day at least 1 week before the experimental day.)

Participants

A total of 48 participants were recruited via the King's College e-mail lists. Inclusion criteria were: age between 21 and 50 years, previous cannabis use ≥1. Detailed screening was performed 1–2 weeks before the experimental session. In addition to clinical examination, the following screening tools were used: The MINI-SCID, The Michigan Alcohol Screening Test and The Drug Addiction Screening Test (Gavin et al., 1989; Selzer et al., 1975; Spitzer et al., 1992). Exclusion criteria were: current pregnancy, a history of mental illness, drug or alcohol dependence (excluding nicotine), current or past severe medical disorders or a history of major mental illness in a first-degree family member. Previous alcohol and drug use were recorded and a urine drug screen was carried out. Participants were asked to avoid alcohol (for 24 h) and drugs (for 1 week) before, and to abstain from driving for 24 h after the experimental session. Pregnancy tests and urine drug tests were used on each study visit to exclude recent drug use or pregnancy in women. Experimental sessions began between 9–10 am and were complete by 4–5 pm. Participants received a brief clinical examination prior to discharge, a 'check-up' phone call the following day and were reimbursed for their time.

Pharmaceuticals

Cannabidiol (2×300 mg capsules) and matching placebo were obtained from STI Pharmaceuticals UK. Synthetic Δ9-THC was supplied by Δ9-THC Pharm GmbH (Frankfurt am Main, Germany) and prepared as 1 mg/mL vials for IV injection, by Bichsel Laboratories (Interlaken, Switzerland) as previously described (Naef et al., 2004). After dilution in normal saline, preparations for injection contained 1.5% (v/v) ethanol absolute. Sterile cannulae were inserted into veins in the antecubital fossa of

both arms: one for administration of Δ9-THC and one for plasma sampling. Δ9-THC was administered in 1 mL/min pulses over a period of 10 mins (total dose 1.5 mg). Blood samples were taken at 1 h, 2 h, 3 h 45 min (5 min post-Δ9-THC), 4 h 10 min (30 min post-Δ9-THC) and 5 h (80 min) post capsule. Doses of oral CBD and IV Δ9-THC were selected on the basis of previous studies (Bhattacharyya et al., 2010; D'Souza et al., 2004; Morrison et al., 2009; Zuardi et al., 2006). Capsules (placebo/CBD) were administered 3 h 30 min prior to IV Δ9-THC challenge, based on the available (albeit limited) knowledge regarding the pharmacokinetics of CBD (Bhattacharyya et al., 2010).

Psychopathological and cognitive measures

Baseline predictive instruments

Prior to the experimental session, participants completed the following questionnaires online: the Green et al. Paranoid Thoughts Scale (GPTS) Part B, which provides a measure of trait paranoia (Green, Freeman, Kuipers, Bebbington, Fowler, Dunn, and P. A. Garety, 2008); the Cannabis Experiences Questionnaire (CEQ), which quantifies psychotic/dysphoric experiences following recreational cannabis use (Barkus and Lewis, 2008); and the Schizotypal Personality Questionnaire (SPQ) (A. Raine, 1991). This permitted assessment of whether measures of 'psychosis-proneness' differed between the two groups. Participants also completed the Wechsler Test of Adult Reading (WTAR), which provides an estimate of IQ (Wechsler, 2001).

Experimental measures

In Table 2.1, the time course of events on the experimental day is illustrated.

Table 2.1. The time-course of the experimental day. Participants were instructed to report/score their experience based upon the peak intensity within the time-window since the previous drug administration (highlighted in bold).

Table 1. The time-course of the experimental day. Participants were instructed to report/score their experience based upon the peak intensity within the time-window since the previous drug administration (highlighted in bold).

Time (hours)	Experimental day
0h00min	**Oral CBD/Placebo administration**, Urinary drug screen
1h00min	Blood sampling CBD
2h00min	Blood sampling CBD
2h20min-2h25min	Post-tablet HVLT
2h25min-2h30min	Post-tablet Digit symbol recoding task
2h30min-2h35min	Post-tablet Digit span forward & reverse
2h35min-2h45min	Post-tablet NAB-Mazes
2h45min	Post-tablet HVLT-recall
Up to 3h00min	Post-tablet PANSS
3h00min-3h10min	Post-tablet Psychological Scales: uMACL, SSPS, BAI, SAM
3h30min-3h40min	**THC-infusion**
3h45min	Blood sampling CBD, THC
4h10min	Blood sampling CBD, THC
4h30min-4h35min	Post-THC HVLT
4h35min-4h40min	Post-THC Digit symbol recoding task
4h40min-4h45min	Post-THC Digit span forward & reverse
4h45min-4h55min	Post-THC NAB-Mazes
4h55min	Post-THC HVLT-recall
5h00min	Blood sampling CBD, THC
Up to 5h20min	Post-THC PANSS
5h20min-5h30min	Post-THC Psychological Scales: uMACL, SSPS, BAI, SAM
6h30min	Discharge

Psychopathology

Under CBD/placebo and Δ9-THC conditions, participants were instructed to report/score their experience based upon the peak intensity within the time-window since the previous drug administration.

Positive psychotic symptoms

The positive psychotic dimension was assessed using two instruments: the PANSS (Kay et al., 1987) (Appendix I) as described previously (Morrison et al., 2009), and The State Social Paranoia Scale (SSPS) (Freeman et al., 2007) (Appendix II). The PANSS was developed for schizophrenia research and consists of a positive subscale (seven items: delusions, conceptual disorganisation, hallucinations, hyperactivity, grandiosity, suspiciousness and hostility), a negative subscale and a general subscale. Items are rated from 1–7 (absent–severe), thus the range on the positive subscale is 7–49. There is a wide inter-individual variation in PANSS positive scores following Δ9-THC and, as a group, positive symptoms are modest compared with acute schizophrenia. In earlier studies approximately 35–50% of healthy participants showed changes of ≥3–4 points (D'Souza et al., 2004; Morrison et al., 2009). The SSPS is a participant-rated instrument consisting of 10 persecutory items (e.g. 'Someone wanted me to feel threatened'), embedded within neutral and positive items. Responses are rated 1–5 (do not agree–totally agree). The SSPS has excellent internal reliability, adequate test-retest reliability, convergent validity with both independent interviewer ratings and self-report measures, and divergent validity with regard to measures of positive and neutral thinking (Freeman et al., 2007).

Affect

The University of Wales Mood Adjective Checklist (UMACL) (Appendix III) was used to assess affect (Matthews et al., 1990). The UMACL is sensitive to change in the three major dimensions of affect: Hedonic Tone (pleasure–displeasure); Energetic Arousal (awake–tiredness); and Tense Arousal (tension–relaxation). On each dimension, participants rated their level of agreement with four positive and four negative adjectives. Scores within each dimension were summed to give a value between -12 and 12, as described previously (Morrison et al., 2009).

Cognition

Three of the four tasks that were employed make up part of the MATRICS Consensus Cognitive Battery (MCCB, PAR, Inc FL 33549) (exception: Digit span). Alternative versions of each task were used across the three different conditions, (baseline, post-capsule, post-Δ9-THC), except for symbol-coding. All participants encountered each version in a consistent order. For each of the three conditions, cognitive tasks were presented in the following sequence (under Δ9-THC conditions, cognitive testing began at 40 min post-Δ9-THC injection).

The Hopkins Verbal Learning Task-Revised (verbal learning and memory)

In the Hopkins Verbal Learning Task-Revised (HVLT-R) (Appendix IV), participants are tested in their immediate recall of 12 words (nouns from three taxonomic categories) after each of three learning trials. Here, delayed recall was assessed 20–25 min after the final learning trial.

Symbol coding (processing speed)

This is a timed pencil-and-paper task in which participants are required to translate a symbol into a corresponding digit (1–9), whilst a reference key of symbol/digit pairs remains visible (Appendix V).

Digit-span forward and reverse (working memory)

The digit span task (forward-condition) (Appendix VI) evaluates the capacity of working memory. Participants are tested for immediate recall of a sequence of digits; and given two attempts at each level of difficulty. In the reverse digit span condition, participants are required to recall the sequence in the reverse order, which places additional processing demands on working memory.

Neuropsychological Assessment Battery mazes (planning and organisational abilities)

In the Neuropsychological Assessment Battery (NAB) mazes (Appendix VII), participants are scored on a composite measure of accuracy and speed in a series of seven progressively more difficult maze-tracing tasks. Since only two equivalent versions are available, this task was only presented at the post-capsule and post-Δ9-THC time-points.

Statistical analyses

All analyses were performed in SPSS 17.0 (SPSS Inc., Chicago). PANSS and SSPS data did not have a normal distribution and were analysed after log transformation as described previously (Kleinloog et al., 2012). In addition, for the PANSS we followed the approach of D'Souza and colleagues, which is to categorise clinically significant psychosis as increases from baseline of ≥3 points (D'Souza et al., 2005): thereafter the difference in the frequency of clinically significant Δ9-THC-evoked psychotic reactions between the CBD and placebo groups was analysed using Pearson's Chi-square. Normally distributed data were analysed by a general linear model (GLM), specifically repeated-measures ANOVA. The within-groups factor was CONDITION (1. Baseline 2. Post-capsule 3. Post-Δ9-THC). The between-groups factor was pre-treatment GROUP (1. CBD 2. Placebo). Greenhouse–Geisser statistics were used in cases where sphericity assumptions were violated. Post-hoc analyses were performed with Bonferroni correction. Relationships between psychosis scores and cognitive data were analysed using Spearman's rank correlation coefficient. Significance was accepted at p values <0.05. All comparisons were two-tailed.

Results

In total, 48 subjects completed the experimental protocol (Placebo group n=26; CBD group n=22). In three subjects, failure of cannulation prevented the administration of Δ9-THC, and data acquired up to that point were not used in any of the analyses. The two groups were adequately matched for demographic variables, baseline measures of 'psychosis-proneness' and previous drug use (Table 2.2). Previous cannabis exposure between the two groups was not significantly different whether data were analysed by comparing means (p=0.76) or ranks (p=0.98).

Table 2.2. Sample characteristics at baseline. The two groups (CBD & placebo) were adequately matched for demographic variables, 'psychosis-proneness' as indexed by the SPQ (Schizotypal Personality Questionnaire;), CEQ (Cannabis Experiences Questionnaire), Green et al. Paranoia Scale, BMI (Body Mass Index), and for previous illicit drug use. There was a trend for higher trait paranoia in the CBD pre-treated group.

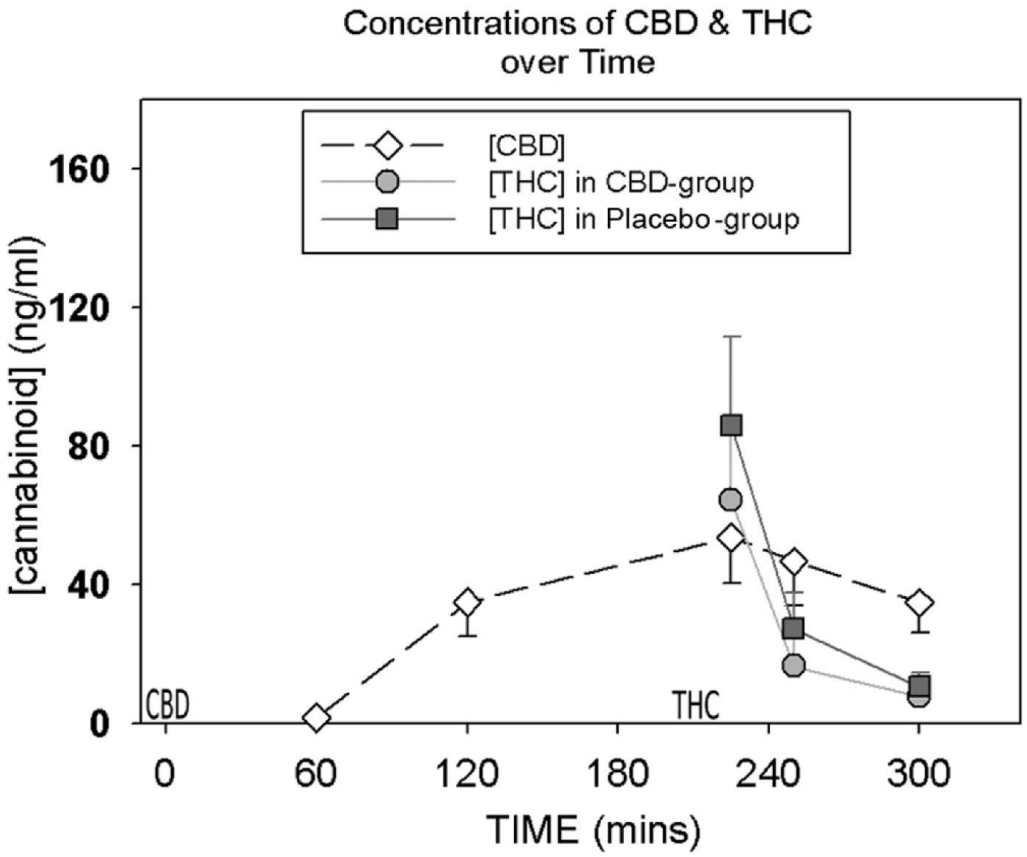
Table 2. Sample characteristics at baseline. The two groups (CBD & placebo) were adequately matched for demographic variables, 'psychosis-proneness' as indexed by the SPQ (Schizotypal Personality Questionnaire;), CEQ (Cannabis Experiences Questionnaire), Green et al. Paranoia Scale, BMI (Body Mass Index), and for previous illicit drug use. There was a trend for higher trait paranoia in the CBD pre-treated group.

Variable	Placebo group	CBD group	*p*
Age (years)	26 (±4)	25 (±3)	ns
Sex ratio (m:f)	14:12	13:9	ns
BMI	25 (±5)	25 (±4)	ns
Wechsler Test of Adult Reading	45.2 (±3.2)	44.3 (±3.4)	ns
SPQ (Total)	11.1 (±7.0)	12.1 (±11.2)	ns
CEQ (paranoia/dysphoria)	43.0 (±9.1)	42.8 (±10.4)	ns
The Green Paranoia scale	19.3 (±5.0)	23.7 (±10.2)	0.08
Previous cannabis use (episodes)	118 (±218)	137 (±234)	ns
Age at first cannabis use	16 (±2)	17 (±2)	ns
Previous drug use (Yes)			
'Ecstasy'	62.5%	48%	ns
Cocaine	54%	40%	ns
'LSD'	21%	20%	ns
Ketamine	21%	32%	ns
Amphetamines	13%	16%	ns
Mephedrone	17%	36%	ns

Pharmacokinetics

The plasma concentrations of CBD and Δ9-THC over time are shown in Figure 2.1. Plasma concentrations of CBD were highest at the 3 h 45 min testing point, before beginning to decrease. Δ9-THC concentrations were not significantly different between the group pre-treated with CBD and the group pre-treated with placebo at 5 min (p=0.5), 30 min (p=0.5) and 80 min (p=0.6) post-Δ9-THC administration.

Figure 2.1. Plasma cannabinoid concentrations (mean±SEM). Oral CBD (600 mg) was administered at 0 min. Δ9-THC (1.5 mg) was administered by slow IV injection from 210–220 min. In the CBD pre-treated group and the placebo pre-treated group, differences in plasma Δ9-THC concentrations at three successive sampling points were not statistically significant. With respect to Δ9-THC administration, plasma [Δ9-THC] was assayed at 5, 30 and 80 min post-injection.



Positive psychotic symptoms

PANSS-positive scores

There was a main effect of CONDITION (F=27.9, p<0.000), but no effect of GROUP (F=1.7, p=0.19) and no interactive GROUP×CONDITION effect (F=2.28, p=0.14) (Figure 2.2). In the placebo group, PANSS positive scores, (mean±sd) increased by 2.4 (±3.1) points following Δ9-THC, compared with 1.2 (±1.8) in the CBD group, a non-significant difference (t=1.5, p=0.15) (Figure 2). Clinically significant positive symptoms following Δ9-THC, defined as an increase in PANSS positive scores of ≥3 points, were more common in the group pre-treated with placebo (11 of 26 cases) compared with the group pre-treated with CBD (3 of 22 cases), (χ^2=4.74, p<0.05) (Table 2.3).

Figure 2.2. Pre-treatment with Cannabidiol, CBD (600 mg po) versus placebo reduced IV Δ9-THC (1.5 mg) elicited increases in PANSS positive scores (mean±SEM), but between group differences did not reach statistical significance (*t*=1.5, *p*=0.15).

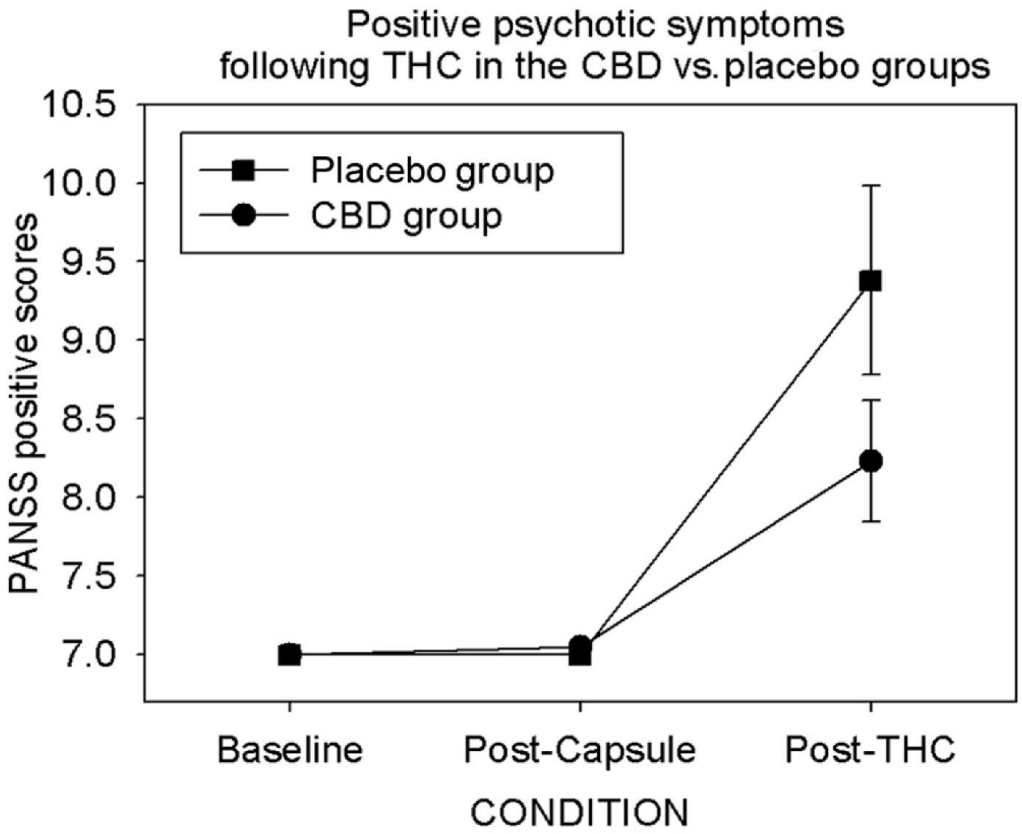


Table 2.3. Pre-treatment with cannabidiol, CBD (600 mg po) reduced the odds of developing a clinically significant acute psychotic reaction to IV Δ9-THC (1.5 mg), defined as a ≥3-point increase from baseline on the PANSS positive subscale.

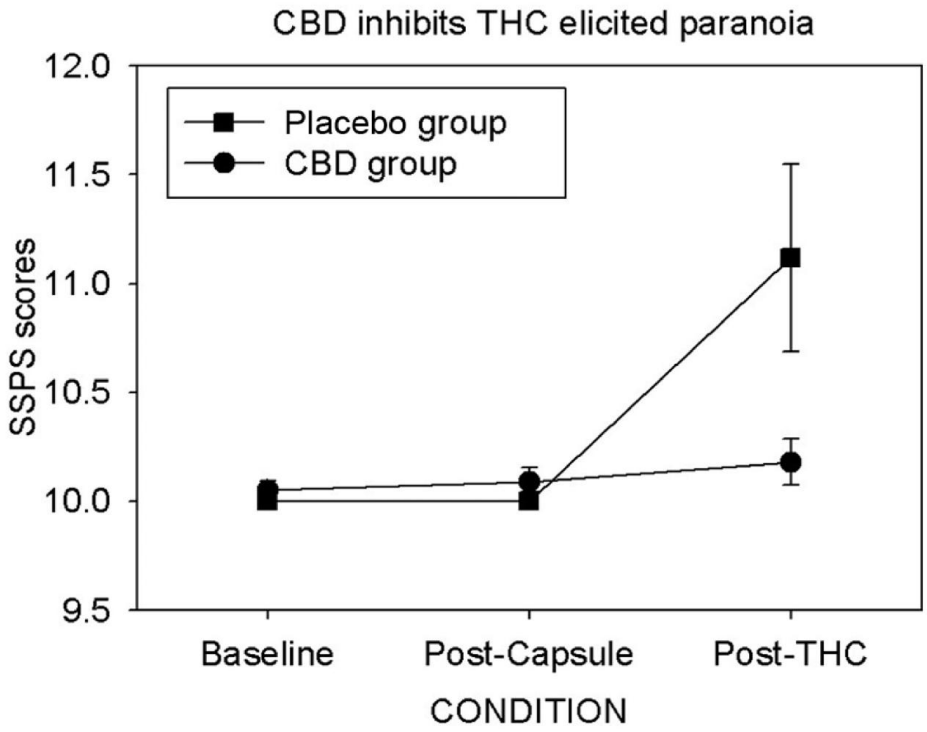
Table 3. Pre-treatment with cannabidiol, CBD (600 mg po) reduced the odds of developing a clinically significant acute psychotic reaction to IV THC (1.5 mg), defined as a ≥3-point increase from baseline on the PANSS positive subscale.

THC psychosis	Pre-treatment		
	Placebo group		CBD group
No	Count;	15	19
	Expected count	18.4	15.6
Yes	Count;	11	3
	Expected count	7.6	6.4
Pearson Chi-Square=4.74, $p<0.05$ (0 cells have expected count less than 5)			
Event rate (psychosis)		42%	14%
Odds of psychosis		0.73	0.16
Absolute risk reduction		28%	
Relative risk		0.33	
Relative risk reduction		67%	
Odds ratio		0.22	

SSPS scores

There was a main effect of CONDITION (F=7.5, p<0.005), but no effect of GROUP (F=2.5, p=0.12). There was a CONDITION×GROUP interaction (F=4.7, p<0.05) (Figure 2.3). The increase in SSPS scores post-Δ9-THC, with respect to baseline, was greater in the placebo versus the CBD group (t=2.28, p<0.05).

Figure 2.3. Pre-treatment with cannabidiol, CBD (600 mg po) inhibited IV Δ9-THC (1.5 mg) evoked paranoia, as measured by the SSPS (mean ±SEM). The increase in SSPS scores [post-Δ9-THC *minus* baseline] was greater in the placebo versus the CBD group (t=2.28, p<0.05).



Affect

Hedonic tone

There were no main effects of CONDITION (F=1.5, p=0.23), GROUP (F=0.001, p=0.98) and no interactive CONDITION×GROUP effects (F=0.23, p=0.74).

Energetic arousal

There was a main effect of CONDITION (F=19.2, p<0.000) but no effect of GROUP (F=0.07, p=0.80) and no interactive CONDITION×GROUP effects (F=1.32, p=0.23). Energetic arousal decreased in the CBD group following the administration of CBD (p<0.01), whereas subsequent decreases following Δ9-THC were not significant (p=0.13). Energetic arousal also decreased in the placebo group, at the level of a trend following the administration of placebo (p=0.08), whereas subsequent decreases following Δ9-THC were not significant (p=1.00).

Tense arousal

There was a main effect of CONDITION (F=28.5, p<0.000) but no effect of GROUP (F=0.003, p=0.98) and no interactive CONDITION×GROUP effects (F=0.58, p=0.50). Tense arousal increased following the administration of Δ9-THC in both groups (CBD group, p<0.005, placebo group, p<0.000).

Cognition

Scores on the cognitive battery at baseline, post-CBD/placebo, and post-Δ9-THC are shown in Table 2.4.

Table 2.4. Under Δ9-THC (IV 1.5 mg) conditions, cognitive performance was generally poorer, except for the Symbol coding and NAB-MAZES tasks. Δ9-THC-elicited deficits in delayed recall were inhibited by CBD (600 mg po).

Table 4. Under THC (IV 1.5 mg) conditions, cognitive performance was generally poorer, except for the Symbol coding and NAB-MAZES tasks. THC-elicited deficits in delayed recall were inhibited by CBD (600 mg po).

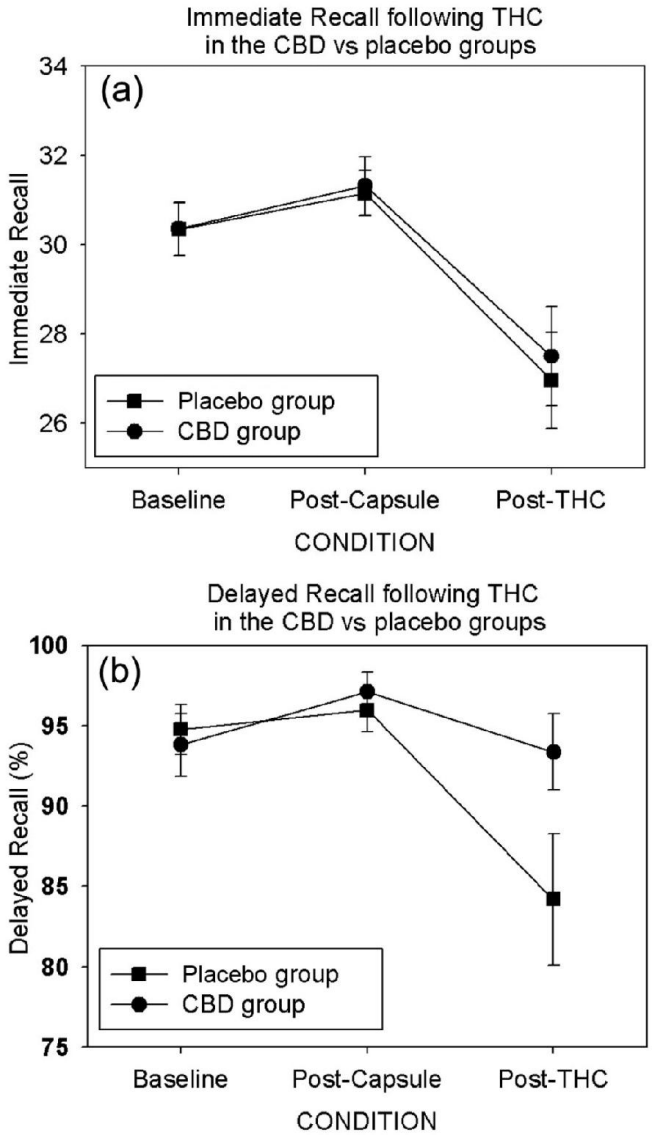
Cognitive test	Placebo group			CBD group			Condition × Group
	Condition			Condition			
	Base	PLC	THC	Base	CBD	THC	
Immediate recall	30.4 (±3.0) F=12.6, p<0.000	31.2 (±2.6)	27.0 (±5.5)	30.4 (±2.8) F=10.5, p<0.005	31.3 (±3.0)	27.5 (±5.2)	F=0.92, p=0.88
Delayed recall	94.8% (±7.9%) F=7.7, p<0.01	96.0% (±7.0%)	84.2% (±20.9)	93.8% (±9.1%) F=1.5, p=0.2	97.1% (±5.8%)	93.4% (±11.1)	F=3.26, p=0.058 Baseline - THC t=2.39, p<0.05
Symbol coding	67.7 (±9.2) F=4.4, p<0.01	70.1 (±9.8)	72.9 (±14.6)	67.6 (±10.4) F=6.7, p<0.01	70.7 (±11.7)	74.6 (±16.1)	F=0.53, p=0.98
Digit span forward	7.5 (±1.2) F=6.1, p<0.005	7.5 (±1.2)	6.6 (±1.2)	7.4 (±1.2) F=2.6, p=0.09	7.7 (±1.1)	7.1 (±1.5)	F=1.24, p=0.30
Digit span Reverse	5.9 (±1.2) F=5.6, p<0.01	6.00 (±1.2)	5.2 (±1.5)	5.7 (±1.4) F=4.1, p<0.05	6.1 (±1.3)	5.2 (±1.4)	F=1.53, p=0.88
NAB-MAZES	– F=1.1, p<0.3	22.6 (±4.1)	21.8 (±3.8)	– F=1.3, p=0.3	23.9 (±2.3)	23.2 (±2.8)	F=0.015, p=0.90

The Hopkins Verbal Learning Task

Immediate recall

There was a main effect of CONDITION (F=22.64, p<0.000) but no effect of GROUP (F=0.079, p=0.78) and no interactive CONDITION×GROUP effects (F=0.92, p=0.88). Immediate recall was poorer following Δ9-THC, regardless of group. Post-hoc analysis revealed differences between post-Δ9-THC and baseline performance, significantly in the placebo group (p<0.005), and at the level of a trend in the CBD group (p=0.06). Differences between post-Δ9-THC and post-capsule performance were significant in the CBD group (p<0.000) and the placebo group (p<0.005). Following Δ9-THC, immediate recall was 2.9 (±5.3) and 3.6 (±4.5) items fewer in the CBD and placebo groups, respectively, compared with baseline, a non-significant between-groups difference (p=0.6), (Figure 2.4(a)).

Figure 2.4. (a) Immediate recall in the HVLT-R (mean±SEM) was poorer following IV Δ9-THC (1.5 mg), in both the placebo and CBD (600 mg po) pre-treated groups. (b) Delayed Recall was poorer following Δ9-THC in the placebo but not the CBD pre-treated group. Relative to baseline, performance under Δ9-THC was poorer in the placebo compared to the CBD group (t=2.39, p<0.05). HVLT-R, The Hopkins Verbal Learning Task-revised.



Delayed recall

There was a main effect of CONDITION (F=7.25, p<0.005), but no effect of GROUP (F=1.75, p=0.19). There was a trend towards a CONDITION×GROUP interactive effect (F=3.26, p=0.058). Post-hoc analysis in the placebo-group revealed differences

between post-Δ9-THC and baseline (p<0.05) and between post-Δ9-THC and post-capsule performance (p<0.05). Corresponding analyses in the CBD group were p=1.0 and p=0.6, respectively. Following Δ9-THC, delayed recall decreased from baseline by 10.6% (±18.9%) in the placebo group and by 0.4% (±9.7) in the CBD group, a significant between-groups difference (t=2.39, p<0.05), (Figure 2.4(b)).

A posteriori, we explored if there were relationships between impaired delayed recall and positive psychotic symptoms, post Δ9-THC. In the placebo group, poorer delayed recall was related to the magnitude of PANSS-positive symptoms, at the level of a trend (Spearman's rho=0.3, p=0.09). The relationship between poorer delayed recall and higher scores on the SSPS was stronger and reached significance (Spearman's rho=0.5, p<0.05); corresponding findings in the CBD group were -0.3, p=0.9 and 0.5, p<0.05).

Symbol coding

There was a main effect of CONDITION (F=11.12, p<0.000) but no effect of GROUP (F=0.003, p=0.98) and no interactive CONDITION×GROUP effects (F=0.53, p=0.82). Performance improved in both groups from the baseline condition to the post-Δ9-THC condition, (CBD group p<0.01; placebo group p<0.05).

Digit-span forward

There was a main effect of CONDITION (F=7.38, p<0.005) but no effect of GROUP (F=0.44, p=0.51) and no interactive CONDITION×GROUP effects (F=1.24, p=0.30). Post-hoc analysis in the placebo group revealed significant differences between digit-span performance in the post-Δ9-THC condition compared with both the baseline (p<0.05) and post-capsule conditions (p<0.05). Corresponding post-hoc analyses in the CBD-group were p=1.00 and p=0.08, respectively.

Digit-span reverse

There was a main effect of CONDITION (F=9.46, p<0.000) but no effect of GROUP (F=0.000, p=0.99) and no interactive CONDITION×GROUP effects (F=1.53, p=0.86). Post-hoc analysis in the placebo group revealed differences between reversed digit-span performance in the post-Δ9-THC condition compared with the baseline (p=0.08)

and post-capsule conditions (p<0.05). Corresponding post-hoc analyses in the CBD-group were p=0.5 and p<0.01, respectively.

Mazes

There were no main effects of CONDITION (F=2.1, p=0.15), GROUP (F=2.4, p=0.13) and no interactive CONDITION×GROUP effects (F=0.015, p=0.90). Numerical differences between groups post-Δ9-THC compared with baseline were not different (t=0.13, p=0.9).

Discussion

Our major findings are that pre-treatment with CBD inhibited Δ9-THC-induced paranoia and inhibited the detrimental effects of Δ9-THC on episodic memory. In addition, CBD decreased the proportion of participants who experienced clinically significant acute Δ9-THC psychosis.

Cannabinoids and psychosis

The majority of community-based studies that have addressed the issue of specific cannabinoid components and psychosis have proposed that cannabis products lacking CBD are more psychotogenic than products that contain CBD (Di Forti et al., 2009; Morgan and Curran, 2008; Schubart et al., 2011); (but see (Morgan, Schafer, et al., 2010)). The findings in the present study provide strong support for this idea. Here, on the PANSS (an investigator-rated scale), clinically significant Δ9-THC psychosis was less likely under CBD versus placebo conditions, and on the SSPS (a participant-rated scale) Δ9-THC-induced paranoia was inhibited under CBD conditions. It is notable that there was a trend for higher *trait* paranoia in the CBD compared with the placebo group, suggesting that the CBD group might have been more prone to paranoia *at baseline*. Post-Δ9-THC however, there was no apparent rise in paranoia in the CBD group, whereas by way of contrast, the placebo group reported significant paranoid symptoms.

Some caution is required, however, with regard to scores on the PANSS positive scale. Although the mean PANSS positive score in the CBD group was less, differences did not reach statistical significance. Lack of statistical power may be important, but it is also

clear that CBD (in so far as it was administered here) does not completely abolish Δ9-THC-induced positive psychotic symptoms.

Cannabinoids and memory

Cognitive performance was poorer following Δ9-THC specifically in the domains of working and episodic memory, which is in keeping with previous reports (reviewed in (Ranganathan and D'Souza, 2006; Solowij and Michie, 2007). Here, pre-treatment with CBD 'protected' episodic memory from the impact of Δ9-THC, whereas working memory remained 'vulnerable' to a similar degree.

This result is in broad agreement with a study carried out by Morgan and Curran: volunteers were assessed at home under the influence of their own chosen type of cannabis, a sample of which was subsequently tested for Δ9-THC and CBD content; higher levels of CBD in the cannabis used appeared to protect against impairments in immediate and delayed prose recall (Morgan, Schafer, et al., 2010). The reason for the differences with regard to immediate recall is unknown, but may stem from the different tasks employed.

Here there were marked performance deficits post-Δ9-THC in three tests which require pre-frontal resources: immediate recall, digit-span forward and digit-span back. CBD did not appear to attenuate Δ9-THC-induced deficits in any of the three tasks. This contrasted with the protective effect of CBD on delayed recall and paranoid symptoms. It is also notable that Δ9-THC-induced impairment in delayed recall and Δ9-THC-induced paranoia were correlated, and it is feasible that both measures load onto a common factor.

Mechanisms

Molecular neuropharmacology

The molecular neuropharmacology of Δ9-THC is well understood: partial-agonism at CB_1 receptors. Although this may be the most relevant pharmacological property of Δ9-THC, many other actions have been identified including agonism of the orphan receptor GPR55, decrease uptake of adenosine, reduced conductance of ligand-gated ion channels of 5-HT_3, activation of PPAR-γ, increased uptake of noradrenaline and

decreased uptake of 5-HT (for review see Pertwee, 2008). For CBD, the picture is more obscure. In an attempt to shed some clarity on the issue, McPartland and colleagues (2014) performed a systematic review of the various pharmacological actions of CBD. Out of eight CBD efficacy studies at the CB1 receptor found no response while the other two found opposing and weak effects at high concentrations. Also, CBD has very poor affinity to the CB1 receptor after pooling the results of fifteen studies. However, six studies found CBD to be a potent antagonist of CB1 agonist at very low concentrations. Put together these results indicate that CBD acts via an indirect mechanism to inhibit Δ9-THC and other agonists compared to a classical antagonist which has affinity for the orthosteric site (McPartland et al., 2014). CBD also has many other pharmacological actions. Five studies found that CBD inhibits anandamide breakdown by FAAH and four studies found that it inhibits reuptake of anandamide via a putative transporter. CBD agonises orphan receptors GPR18 and antagonises GPR55, both which opposes the actions of Δ9-THC. Twelve studies found CBD to activate transient receptor potential ion channels (e.g. TRPV1, TRPV2, TRPA1, TRPM8). Seven studies have shown that CBD can modulate intracellular calcium levels, a mechanism which also triggers anandamide production. It has also been found that CBD inhibits the reuptake of adenosine (3 studies), positive allosteric modulation of α3 glycine (3 studies), PPAR-γ activation (5 studies), and reduces production of nitric oxide (15 studies. A further twelve studies support the notion of CBD as an allosteric modulator of receptor systems such as α_1-adrenoceptors, dopamine D_2, $GABA_A$, μ- opioid and δ-opiod receptors. It remains unclear as to which of these pharmacological actions are involved in the inhibitory actions of CBD on Δ9-THC and further in vitro work will be required to identify which action underlies a particular psychopharmacological effect, at the systems and the behavioural levels.

Systems pharmacology

How Δ9-THC impacts upon episodic memory is reasonably well understood. Episodic memory depends upon the integrity of hippocampal circuitry. Numerous animal studies have shown that CB_1 agonists disrupt processes within the hippocampus that are believed to be at the heart of learning and memory – network oscillations, neuronal synchrony and plasticity (Fan et al., 2010; Hájos et al., 2000; Holderith et al., 2011; Robbe and Buzsáki, 2009). Recently, CB_1 agonists have become a useful tool in

hippocampal research. This is because CB_1 agonists disrupt synchronicity, without altering the firing rates of individual neurons in the network – a unique property amongst drugs which impact on hippocampal function (Robbe et al., 2006).

The mechanisms underlying the pro-psychotic properties of Δ9-THC are less well understood. Theoretical accounts have invoked excessive, pathological dopamine release (Kuepper et al., 2010; Murray et al., 2007), but experimental support for this has been weak (Barkus et al., 2011; Bossong et al., 2009; D'Souza, Braley, et al., 2008; Kleinloog et al., 2012; Stokes et al., 2009) (but see (Liem-Moolenaar et al., 2010)). Other accounts have focussed on disrupted network oscillations (Sewell et al., 2009). Here the experimental evidence has been stronger (Morrison et al., 2011; Stone et al., 2012) but remains at an early stage.

In the present dataset, we were interested by the apparent relationship between Δ9-THC psychosis and Δ9-THC-elicited impairments in episodic memory. However, the presence of such a relationship was not hypothesised a priori, and replication is required.

Strengths and limitations

In laboratory-based pharmacological studies, pure synthetic preparations can be administered at a set dose under controlled conditions. This is particularly relevant for cannabinoid studies because 'street cannabis' contains a multitude of other molecules, many of which are known to be pharmacologically active. One example is Δ^9-Tetrahydrocannabivarin (Δ9-THCV), a CB_1 receptor antagonist at low doses, an agonist at higher doses (Pertwee, 2008). Compared with 'street cannabis', pure synthetic preparations are ideal for studying the behavioural pharmacology of specific cannabinoid molecules, because pharmacodynamic and pharmacokinetic influences from other constituents can be disregarded from the outset. A limitation in the present study is that only one dose of CBD was investigated. Future studies could examine if higher CBD doses, or indeed extended dosing over several days, produce stronger 'protective effects', or if protection extends to additional domains such as working memory.

Conclusions

Previous epidemiological and experimental studies have suggested that cannabis products lacking CBD are more psychotogenic than products containing CBD. The findings here provide strong support for this view. Under controlled experimental conditions, CBD decreased Δ9-THC-elicited positive psychotic symptoms and 'protected' hippocampal-dependent memory from the impact of Δ9-THC.

Post-article comments

In this study, although CBD significantly inhibited Δ9-THC-induced paranoia, impairments to delayed verbal recall and reduced the proportion of clinically significant psychosis; CBD did not completely abolish the psychotogenic effects of Δ9-THC nor protects against working memory impairment. Subjectively, all participants experienced intoxication following Δ9-THC, although this was not measured in a standardised way which precludes inferences about the effects of CBD on the subjective effects of IV Δ9-THC. Also, it is not possible to determine if the individuals who experienced psychosis in the CBD group would be protected from a higher dose of CBD. Alternatively, it is possible that passed a certain dose of Δ9-THC, CBD becomes ineffective. To clarify this, future studies would benefit from exploring a range of doses for both Δ9-THC and CBD.

An interesting finding was that of a trend towards a group difference between the CBD and placebo groups in terms of scores on the Green Paranoid Thoughts scale, where the CBD group scored higher. This has potential implications in terms of the specific protective role of CBD towards paranoid thoughts. A recent study found that individuals predisposed to paranoid thinking were more susceptible to the paranoia inducing effects of Δ9-THC (D. Freeman et al., 2014). Since there was no significant increase in paranoia in the CBD group following THC, in spite of them being slightly more predisposed to begin with, it may suggest that CBD has a particularly strong anti-paranoid effect.

Pleasure is one of the main reasons for the recreational use of cannabis. Although we did not include this data in the manuscript, it was collected for this study. Data was collected on a 5-point likert scale on the item: "This experience is pleasurable". There

was a significant main effect of CONDITION ($F=37.591$, $p<0.001$) but no effect of GROUP ($F=0.118$, $p=0.73$) or a CONDITIONxGROUP interaction ($F=0.065$, $p=0.937$). Post-hoc analysis revealed a significant increase in pleasure scores between post-capsule and post-THC in both the placebo ($p<0.001$) and CBD groups ($p<0.001$), but no difference between the groups at the post-THC time point ($p=0.983$). This indicates that CBD does not significantly affect the pleasurable effects of THC, which could be important from a public health perspective. As the most frequent users grow tolerant to THC and require larger quantities to achieve the same level of intoxication (Desrosiers et al., 2015), this may lead them to consuming cannabis with greater THC content which may subsequently be more harmful (Di Forti et al., 2009, 2013). This finding may suggest that cannabis products with a high THC and high CBD content may still be preferred by frequent users as CBD does not interfere with the pleasurable effects of cannabis, and may be less harmful compared to cannabis containing only THC.

Although Δ9-THC significantly impaired performance on working memory tasks such as forward and reverse digit-span and immediate verbal recall, performance on speed-of-processing and executive function tasks (symbol recoding and mazes task) were not affected. Previous studies have found that some areas of cognition are less impaired by Δ9-THC (Pope et al., 1995; Ramaekers et al., 2006a). These results further strengthen the notion that Δ9-THC impacts on processing speed and executive function less compared to other cognitive domains.

The choice to exclude participants who were currently or previously dependent on cannabis may have impacted the result of this study. Previous studies have shown that frequent users are more resilient to the acute cognitive (Hart et al., 2010) and psychotogenic (D'Souza, Braley, et al., 2008) effects of Δ9-THC. This is likely due to tolerance built up from frequent and repeated use of cannabis, as well as downregulation of the brains CB1 receptors (Hirvonen et al., 2012). Conversely, individuals sensitive towards the acute effects of Δ9-THC who also have had unpleasant experiences from cannabis are unlikely to volunteer in research. This may also have confounded the results of this study by including participants who were more resilient towards Δ9-THC. Luckily, both groups were equally matched on the paranoia/dysphoria measure of the CEQ (which indicates previous unpleasant

experiences while using cannabis) as well as number of use occasions, suggesting no group differences in possible resilience towards Δ9-THC.

Apart from only using one dose of CBD being a limitation of this study, so was the oral administration of CBD. Ideally, CBD should have been administered intravenously which would have allowed an interpretation of the CBD/Δ9-THC ratio which is less harmful. In a previous pilot-study, Morrison and colleagues administered 5mg IV CBD before 1.25mg IV Δ9-THC and found that CBD protected against positive psychotic symptoms (Morrison et al., 2010); a CBD/Δ9-THC ratio of 4. However, plasma levels of CBD were stable among participants, and Δ9-THC was administered at peak CBD levels.

Another possible limitation to the study was the use of a between subjects design. Similar to previous studies with IV Δ9-THC (D'Souza et al., 2004; Morrison et al., 2009), there was great individual variability of reactions to Δ9-THC, where just over 40% of participants experienced paranoia and psychosis. To better capture the protective effects of CBD, the same individual would be given the same dose of IV Δ9-THC twice, co-administered with either CBD or placebo. However, the participant number in this study was large enough for significant effects to be found.

Electrophysiological effects of Δ9-THC and CBD

Introduction

As discussed earlier, the two main components of cannabis are Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). It is well established that Δ9-THC is the main cannabinoid responsible for the psychological effects of cannabis, and given in higher doses is able to induce psychotic symptoms and memory impairments similar to that of schizophrenia (D'Souza et al., 2004; Morrison et al., 2009). CBD on the other hand has been shown to reduce the anxiety inducing effects (Zuardi et al., 1982), psychotic symptoms (Morgan and Curran, 2008; Morgan et al., 2012; Englund et al., 2013), and memory impairing effects of Δ9-THC (Morgan, Schafer, et al., 2010; Englund et al., 2013). Δ9-THC acts as a partial agonist at the CB1 receptor, while the pharmacological actions of CBD are less clear and include such pharmacological effects as acting as a $5HT_{1A}$ agonist, adenosine reuptake inhibition, TRPV1 agonist, GRP55 agonist, and causing increased intra-cellular calcium (Izzo et al., 2009). Although these effects are likely to play a part in reducing the negative effects of Δ9-THC, other pharmacological actions are more likely. It is known that CBD has a direct inhibitory effect on CB1 agonists, and in several ways (increased calcium, endocannabinoid reuptake inhibition, inhibition of FAAH) causing increased endocannabinoid levels which may compete with Δ9-THC (Pertwee, 2008).

The CB1 receptors are densely populated in the main areas of the brain related to the effects of cannabis: hippocampus, neocortex, cerebellum and basal ganglia (Glass et al., 1997). The endocannabinoid system regulates synaptic weight in these regions by producing and releasing endocannabinoids from GABAergic and glutamatergic post-synaptic terminals following activation of these terminals (increased intracellular calcium) (Fernandez-Espejo et al., 2009). As the endocannabinoid system is finely tuned to the rhythms of the network, it is likely that exogenous cannabinoids such as Δ9-THC may fail to mimic this effect and instead disrupt the network in a dose dependent manner.

Studies recording neural activity using electroencephalography (EEG) are well equipped to capture changes to precise firing of neural networks as EEG provides

excellent temporal resolution on a millisecond scale. However, the interpretation of EEG is limited as electrical activity is recorded merely from the scalp which makes interpretation of the origins of the oscillations difficult. Furthermore, EEG is vulnerable to interfering artefacts produced by ocular movements and scalp muscles tensions which can distort the recording or make interpretation difficult (Croft and Barry, 2000; De Vos et al., 2010).

Studies from the past decades have highlighted the importance of neural oscillations in higher order functions such as memory, synaptic plasticity, attention, perception, motor control and consciousness (Uhlhaas et al., 2008; Buzsáki and Draguhn, 2004). EEG activity is divided into 5 main frequency bands: Delta (0-3.5Hz), Theta (3.5-7Hz), Alpha (8-13Hz), Beta (14-25Hz) and Gamma (30-200Hz). Network oscillations result from the synchronised firing of large assemblies of neurons, which also constrain the firing of individual neurons to the rhythms of the network (Buzsáki and Draguhn, 2004; Varela et al., 2001). This synchronised activity helps strengthen network connections (long term potentiation) (Pavlides et al., 1988), as well as aiding rapid short (gamma) and long-distance (alpha, beta, theta) communication between brain regions (Kopell et al., 2000; von Stein et al., 2000).

Several animal studies have highlighted the importance of the endocannabinoid system in regulating and maintaining neural oscillations. Such studies benefit from the ability to insert electrodes into specific areas of interest of the brains of the animals or alternatively relevant brain areas may be removed from the animal and tested *in vitro.* Hajos and colleagues showed that gamma oscillations in the CA3 area of the hippocampus were significantly reduced following administration of a selective CB1 agonist. The authors concluded that activation of presynaptic CB1 receptors reduced the power of hippocampal oscillations by exerting inhibition of GABA release (Hájos et al., 2000). In a similar *in vitro* study, release of endocannabinoids interrupted hippocampal theta rhythms in the CA1 region of the hippocampus (Reich et al., 2005). An *in vivo* study in anesthetized freely moving rats showed that administration of the potent CB1 agonist CP55940 significantly reduced theta and gamma power in the hippocampus (Hajós et al., 2008). This effect was associated with reduced auditory sensory gating, a phenomenon which has been linked to abnormal information processing (Freedman et al., 2003), and was blocked by co-administration of a CB1

antagonist. Kucewicz and colleagues measured electrophysiological changes produced by administration of CP55940 while the rats were performing cognitive tasks. They reported a significant decrease in hippocampal theta and co-occurring prefrontal gamma following drug administration. These effects were also correlated with impaired cognitive performance on a spatial working memory task (Kucewicz et al., 2011). Furthermore, a study administering Δ9-THC and CP55940 to rats reported impaired performance during a memory task which was correlated with reduced hippocampal theta but not gamma (Robbe et al., 2006). Reductions in power were also associated with reduced temporal synchronicity, which the authors conclude may underlie the cognitively impairing effects of exogenous cannabinoids.

Most electrophysiological studies of cannabinoids in humans have examined the effects of cannabis (either the acute effects of cannabis or comparing chronic users to non-users) on event related potentials (ERPs). Such studies have consistently shown a reduction of amplitude of ERPs, often in a dose dependent manner, during acute intoxication with Δ9-THC (D'Souza et al., 2012; Spronk et al., 2011; Greenwood et al., 2014; K B E Böcker et al., 2010); the effects were significantly less pronounced in more frequent users (Theunissen et al., 2012; Hart et al., 2010). Two studies further explored the differences between Δ9-THC and combination of Δ9-THC and CBD on ERPs. They showed that CBD had no significant impact on Δ9-THC-induced P300 reductions (Roser et al., 2008), but also that the combination of CBD and Δ9-THC increased the mismatch negativity (MMN) ERP while this was unchanged during Δ9-THC and placebo conditions (Juckel et al., 2007).

Studies investigating differences between users and non-users on EEG power have observed reduced power among users (Skosnik et al., 2006), which also correlated with age of onset of use (Skosnik et al., 2012). Ilan and colleagues measured EEG after participants smoked a standardised cannabis cigarette containing either 0% or 3.45% Δ9-THC. They reported reduced theta power after Δ9-THC compared to placebo across the scalp, as well as reduced alpha reactivity during increased difficulty on the working memory task. These effects coincided with reduced accuracy and slower response times on the working memory task, as well as increased false-positive responses on an episodic memory task (Ilan et al., 2004). In a subsequent study by the same group, EEG effects of low and high dose Δ9-THC (1.8% and 3.6%) co-administered with either low

or high dose CBD (between 0.1-0.4% and greater than 1%) were investigated. Although Δ9-THC reduced alpha, beta, and theta power and impaired working and episodic memory performance following both doses of Δ9-THC, there was no significant protective effect of CBD (Ilan et al., 2005). The dose dependent effect of Δ9-THC on EEG power was further explored by Böcker and colleagues (Koen B E Böcker et al., 2010). They had participants smoke cannabis cigarettes containing 29.3mg, 49.1mg, and 69.4mg Δ9-THC and observed a dose response reduction in theta and beta power. Furthermore, a greater decrease in theta power coincided with slower response times on a working memory task.

As mentioned previously, inhaled or orally administered Δ9-THC suffers from great inter-individual variation in bioavailability (Grotenhermen, 2003), which is why the intravenous route of administration is preferred. In a novel study, Morrison and colleagues explored the effects of intravenous Δ9-THC on EEG power and coherence (a measure of temporal synchronicity between scalp locations) (Morrison et al., 2011). They also measured performance on a working memory task and psychotic symptoms as measured by the PANSS. Δ9-THC significantly increased psychotic symptoms and slowed reaction time compared to placebo, while also significantly reducing theta power, and theta coherence between bi-frontal electrode regions. There was a significant correlation between the reduction in bi-frontal theta coherence and positive psychotic symptoms on the PANSS scale. In a subsequent study, Stone and colleagues explored the relationship between changes to frontal inter-trial coherence (ITC, a measure of synchronised oscillations during the 150ms preceding speech) and psychotic like symptoms following intravenous Δ9-THC. ITC was significantly reduced by Δ9-THC and was correlated with measures of salience and ipseity disturbance (Stone et al., 2012). Studies comparing differences between healthy volunteers and patients with schizophrenia have observed reductions in EEG power and synchronicity, similar to what has been observed in Δ9-THC studies (Ford et al., 2002; Ford and Mathalon, 2008; Uhlhaas et al., 2008).

The human literature on EEG effects of Δ9-THC has consistently observed reductions to theta power and synchronicity, where these reductions correlate with poorer performance on cognitive tasks and increases in psychotic symptoms. In the following study I will explore the potential protective effect of CBD on Δ9-THC-induced changes

to EEG amplitude and coherence. I hypothesise that Δ9-THC will significantly reduce theta amplitude and coherence, and that these reduction will be inhibited by CBD. Furthermore, reduction in theta coherence will correlate with positive psychotic symptoms.

Methods

The study was approved by the Joint Institute of Psychiatry and Maudsley Hospital Ethics Committee. All participants were given at least 24 hours to study the participant information sheet and provided written informed consent. Participants were informed that they could withdraw from the study at any time for any reason and that all information relating to their participation would be kept anonymous. They were informed of potential adverse effects of intravenous Δ9-THC (short-lived feelings of anxiety and psychosis-like symptoms), and that rescue medication (Lorazepam 1-4mg) would be made available in case participants so wished.

Design

This was a randomized, double-blind, placebo-controlled, between subject study in which 600mg oral Cannabidiol or matched placebo as a pre-treatment before intravenous administration of 1.5mg Δ9-THC. The timeline for the study is presented in Table **3.1**. Participants were first invited for a screening visit. During this visit a brief medical screening was performed, participants were evaluated for eligibility, consented to the study and baseline assessment of cognitive and psychopathology scales were performed. At the end of the screening visit the participants were booked in for an experimental session and told to avoid all drug use until the end of the study, and avoid alcohol 24 prior to the experimental session. Participants were asked to have a normal breakfast, but informed that no caffeinated beverages would be allowed on the day. The experimental session started with baseline EEG recordings, following which the participant was administered either placebo or CBD. A 2 hour break followed to allow for absorption of CBD, based on previous pharmacokinetic data (Bhattacharyya et al., 2010; Zuardi et al., 2006). This was followed by the post-capsule EEG recordings, cognitive and psychological assessments. Participants were then infused with intravenous Δ9-THC over 10 minutes, which was followed by post-

Δ9-THC EEG, cognitive and psychological testing. Once all testing had been completed, the participants stayed in the lab until the effects of Δ9-THC had worn off, which took approximately three hours. Participants were followed-up the day after via telephone in order to make sure there were no delayed negative effects of the drug. Only one participant reported mild sedative effects which wore off after a few days.

Table 3.1. Experimental timeline

Screening day	
	Information and Consent
	Medical screening
	Baseline Cognitive and Psychological tests
Experimental day	
	Baseline EEG
0h 0min	Oral CBD 600mg or Placebo
2h 0min	Post-Capsule EEG
2h 30min	Post-Capsule Cognitive and Psychological tests
3h 30min	Intravenous THC 1.5mg (over 10 minutes)
3h 50min	Post-THC EEG
4h 20min	Post-THC Cognitive and Psychological tests
6h 30min	Discharge

Participants

Forty eight healthy male and female participants were enrolled to the study via circular email to King's College staff and students and word of mouth. Participant demographics are presented in Table **3.2**. Three were excluded from the final EEG analysis due to incomplete or noisy EEG recordings. Inclusion criteria were healthy male and female volunteers aged between 21-50 years. Exclusion criteria included

being cannabis naïve, history of mental illness (psychotic disorder, depression, anxiety), past treatment with psychotropic medication, major physical illness, major mental illness in first degree family member, history of alcohol or substance misuse (excluding tobacco) and currently pregnant. Pregnancy tests and urine drug tests were used on each study visit to exclude recent drug use or pregnancy in women. All participants provided written informed consent.

Table 3.2. Participant demographics

Variable	Placebo (N=24)	CBD (N=21)	p
Age (years)	25.7 (±4)	24.3 (±3)	ns
Sex ratio (m:f)	12:12	13:8	ns
BMI	24.8 (±5)	25.1 (±4)	ns
WTAR	45.1(±3)	44.6(±3)	ns
SPQ (total)	11.7 (±7)	12.5 (±11)	ns
CEQ (paranoia/dysphoria)	44.1 (±9)	42.3 (±10)	ns
GPTS	19.4 (±5)	23.1 (±10)	ns
Previous cannabis use	125 (±226)	145 (±243)	ns
Age of first cannabis use	16.3 (±2)	16.6 (±2)	ns
			ns
Previous drug use (Yes)			ns
Ecstasy/MDMA	63.60%	52.40%	ns
Cocaine	59.10%	38.10%	ns
LSD	18.20%	14.30%	ns
Katemine	22.70%	33.30%	ns
Amphetamines	13.60%	9.50%	ns
Mephedrone	18.20%	33.30%	ns

Pharmaceuticals

CBD (2 x 300mg) capsules and matching placebo were provided by STI Pharmaceuticals UK. Synthetic Δ9-THC was acquired from STI Pharmaceuticals UK, via Δ9-THC Pharm GmbH (Frankfurt am Main, Germany) and prepared as 1 mg/mL vials for IV injection, by Bichsel Laboratories (Interlaken, Switzerland). 1.5mg Δ9-THC was diluted in 8.5ml

normal saline in a 10ml syringe. Δ9-THC was administered over 10 minutes with 1ml of the solution being injected each minute.

Cognitive and Psychological measures

Baseline predictive measures

Participants provided demographic information and completed the Green et al Paranoid Thought Scale (part B, trait paranoia) (Green, Freeman, Kuipers, Bebbington, Fowler, Dunn, and P. a Garety, 2008), Schizopytal Personality Questionnaire (SPQ) (a Raine, 1991), Cannabis Experience Questionnaire (CEQ) (Barkus and Lewis, 2008), Wechslers Test for Adult Reading (WTAR) (Wechsler, 2001) at the start of the study. These scales were used to compare the two groups on levels of trait paranoia (GPTS), psychosis-proneness (SPQ), cannabis and drug use history as well as levels of paranoid/dysphoric experiences during cannabis use (CEQ), and levels of pre-morbid IQ. There were no significant differences between the CBD and placebo groups on any of these measures (Table **3.2.**).

N-Back task

The n-back task has been widely used as a working-memory (WM) task in a variety of neuroimaging studies (Owen et al., 2005) (Example playlist in Appendix VIII). The tasks place increasingly greater demand on many aspects of WM as it requires participants to monitor, update, remember and manipulate information as the task becomes more difficult. In this study participants performed the n-back task while EEG was recorded. For the n-back task participants are asked to monitor a series of letters appearing on a computer monitor in front of them, and asked to respond whenever a letter is the same as one presented n trials previously, as quickly as possible. The task starts with the 0-back condition which requires the participant to respond whenever the letter X is presented. This is followed by the 1-back, 2-back and lastly 3-back condition; each subsequent condition being more difficult and requires greater cognitive effort. Participants were sat at eye-level ~66cm from a CRT monitor and instructed to make their responses on a red joy-pad using their right index finger (even for left-handed participants).

Positive And Negative Syndrome Scale (PANSS)

The PANSS is a validated investigator rated measure of positive (delusions, hallucinations, suspiciousness, hyperactivity, conceptual disorganisation, and hostility) negative and general psychotic symptoms used mainly for schizophrenia research (Kay et al., 1987). Each item is scored on a 7 point scale from absent (1) to severe (7). To assist scoring each point level of each item has a description of the characteristics of the scoring. PANSS was assessed at baseline, post-capsule, and post-Δ9-THC. The post-Δ9-THC PANSS was performed after the main psychoactive effects of Δ9-THC had started to wear off, so that possible Δ9-THC-induced feelings of paranoia and anxiety towards the researchers would not lead the participant to withhold information regarding their feeling states. In fact, retrospective accounts from previous studies (Morrison et al., 2009) have indicated that participants may feel distrust towards researchers while under the influence of Δ9-THC, hence reporting no significant change in mood or thoughts.

State Social Paranoia Scale (SSPS)

The SSPS is a 20-item self-rated scale which consists of 10 neutral items and 10 items asking questions relating to state paranoia and persecutory thinking (Freeman et al., 2007). Each item is scored on a 5-point scale ranging from 'do not agree' to 'totally agree'. Participants were asked to rate the scale according to what they had been feeling and thinking within the last 20 minutes. For the Δ9-THC session, participants were asked to rate the scale based on the peak intensity of the Δ9-THC-experience.

Electroencephalography

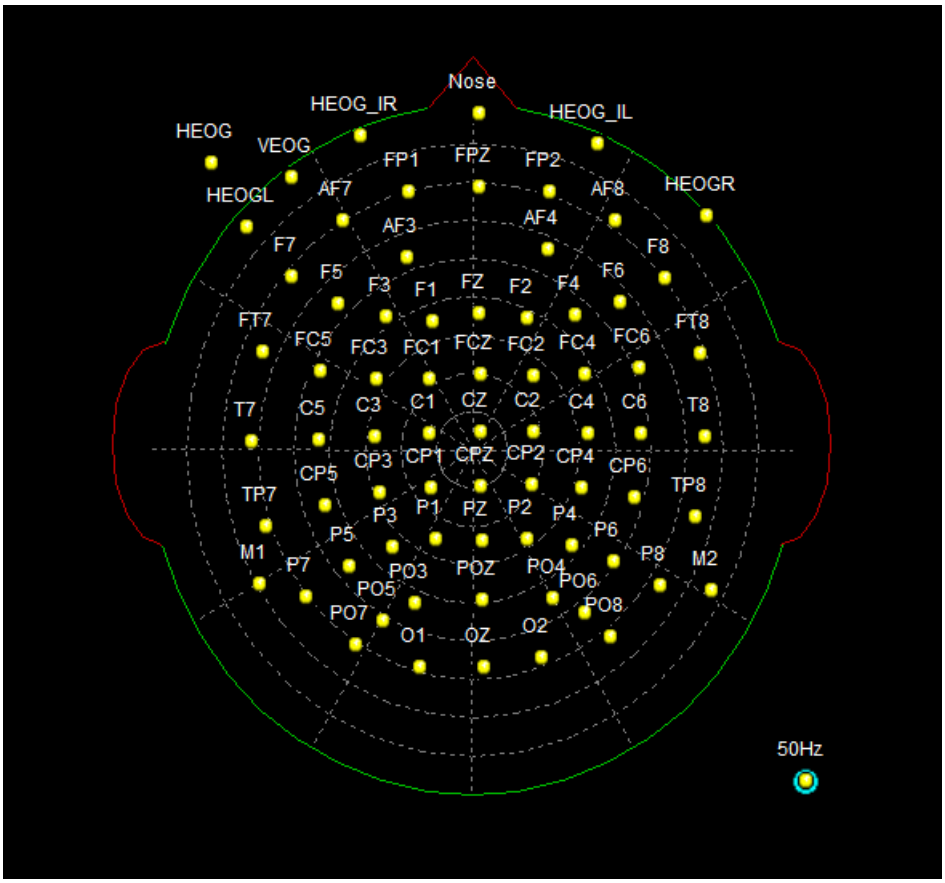
Neuroscan 4.3 was used for all data acquisition and processing in this study. A 64 electrode Quick-Cap Systems (Compumedics) EEG cap was used for the recording of EEG data. The recording reference electrodes were place on both earlobes, data was re-referenced to average mastoid electrodes, and the ground was connected at AFz. Impedances were kept below 10 kΩ by adding additional conductive gel to the electrodes and relocating the participant's hair at the site of the electrode to achieve a better connection. Additional electrodes were placed above and below the left eye of the participants to measure vertical electrooculographic (EOG) activity, as well as

electrodes on the outer canthi to measure horizontal EOG. The EEG was recorded at a 5000 Hz sampling rate, and was down-sampled to 1000 Hz before processing. Major noise to the EEG trace was manually removed, and eye-blinks were corrected using the data from the horizontal and vertical EOG. The data was then baseline corrected and epoched using a 10% Hanning window into 2.048 second sections from 24ms before to 2024ms after each presentation of an n-back letter. Average amplitude was calculated within the frequency bands delta (1-3.5 Hz), theta (3.5-7 Hz), alpha (8-13 Hz), beta (14-25 Hz), gamma (30-40Hz); using Fast Fourier Transform for each of the four n-back conditions. For the amplitude analysis, electrodes were grouped into the following regions: left frontal (LF), right frontal (RF), left central (LC), right central (RC), left temporal (LT), right temporal (RT), left occipito-parietal (LOP), right occipito-parietal (ROP) (see Table 3.3) (for EEG electrode location see Figure 3.1). Electrodes FZ, CZ, and PZ were analysed individually.

Table 3.3. Grouping of electrodes.

Location	Electrodes
Left frontal (LF)	F1, F3, F5, F7, AF3
Right frontal (RF)	F2, F4, F6, F8, AF4
Left central (LC)	C1, C3, FC1, FC3
Right central (RC)	C2, C4, FC2, FC4
Left temporal (LT)	FT7, T7, TP7, CP5, P7
Right temporal (RT)	FT8, T8, TP8, CP6, P8
Left occipito-parietal (LOP)	O1, PO5, PO3, P3, P1
Right occipito-parietal (ROP)	O2, PO6, PO4, P4, P2

Figure 3.1. Location of EEG electrodes.



Coherence refers to a measure of correlation of EEG activity between two scalp locations for a specific frequency band, and consists of a value ranging from 0 to 1 (Pearson's correlation equivalent). For the analysis of coherence, data was transformed to bipolar derivations of pairs of neighbouring electrodes at two locations: Left-frontal (F3/F5) and right-frontal (F4/F6). Coherence was measured for each n-back condition between bi-frontal (F3/F5- F4/F6) electrode pairs.

Statistical analysis

All statistical analyses were performed in SPSS 21 (IBM, N.Y.). Similarly to previous studies (D'Souza et al., 2005; Englund et al., 2013), a clinically significant psychotic reaction was defined as an increase of 3 or more points on the PANSS positive sub-scale. Pearson's Chi-squared test was used to compare group differences in frequency of clinically significant psychosis. Due to floor effects on both the PANSS and SSPS, Friedman's non-parametric test was used to assess differences in scores across sessions. Mann-Whitney U tests were used to analyse differences between CBD and placebo groups. Accuracy and reaction time on the N-back task was assessed using

repeated measures ANOVA, with Session (Baseline, Post-Capsule, Post-Δ9-THC) and Load (0-back, 1-back, 2-back, 3-back) as within subjects factor, and Treatment (CBD, Placebo) as between subjects factor. For EEG amplitude, repeated measures ANOVA were used for each frequency band (delta, alpha, theta, beta, low gamma, high gamma). The within-subject factors were Location (LF, RF, LC, RC, LT, RT, LOP, ROP, FZ, CZ, PZ), Session (Baseline, Post-Capsule, Post-Δ9-THC), working memory Load (0-back, 1-back, 2-back, 3-back), and Treatment (Placebo, CBD) as between-subjects factor. Similarly, repeated measures ANOVA were used for EEG coherence with Session (Baseline, Post-Capsule, Post-Δ9-THC) and working memory Load (0-back, 1-back, 2-back, 3-back) as within-subjects factors, and Treatment (Placebo, CBD) as between-subjects factor. Log-transformations were implemented where data was not normally distributed, and Huynh-Feldt corrected statistics were used where assumptions of sphericity were violated. Correlations between changes to EEG coherence and psychopathology were done using Spearman's rho. The changes in scored on the PANSS, SSPS and EEG coherence were calculated as the difference between post-capsule and post-Δ9-THC testing points. Post hoc analyses and multiple comparisons of correlations were Bonferroni corrected. All analyses were two-tailed and significance levels were accepted at $p<0.05$.

Results

Cognition and psychology

N-back

Accuracy (as a percentage of correct responses) on the N-back task did not significantly change following Δ9-THC (Session: $F=2.261$, $p=0.118$). There was a significant reduction in performance as the task got harder (Load: $F=73.888$, $p<0.001$), and there was a trend towards a Session x Load interaction ($F=2.324$, $p=0.075$). CBD did not affect performance (Treatment: $F=0.662$, $p=0.421$), and there was no significant Session x Treatment effect ($F=1.019$, $p=0.358$) (Figure 3.2).

Reaction time on the n-back task was significantly increased across sessions (Session: $F=6.352$, $p=0.003$). Δ9-THC only increased reaction time on a trend level compared to baseline ($p=0.075$), while this was not significant when comparing to the post-capsule

session (p=0.921). As the task got harder, reaction time significantly increased (Load: F=76.589, p<0.001). CBD did not influence reaction time (Treatment: F=2.366, p=0.133), and there was no Session x Treatment interaction (F=1.326, p=0.272) (Figure 3.3).

Figure 3.2. Percentage correct responses on the N-back task (mean± SEM)

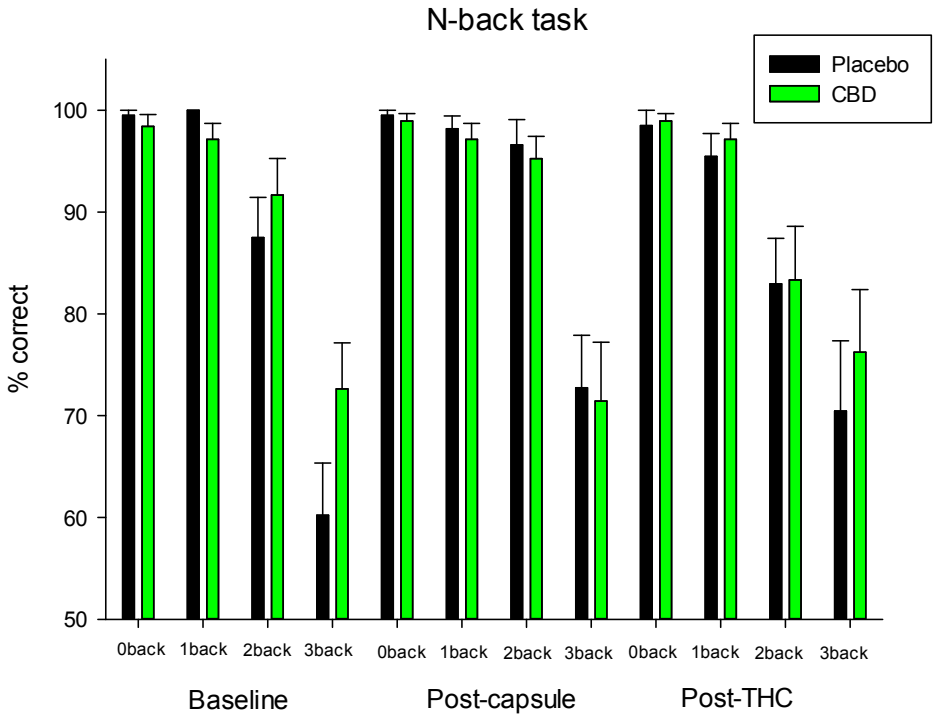
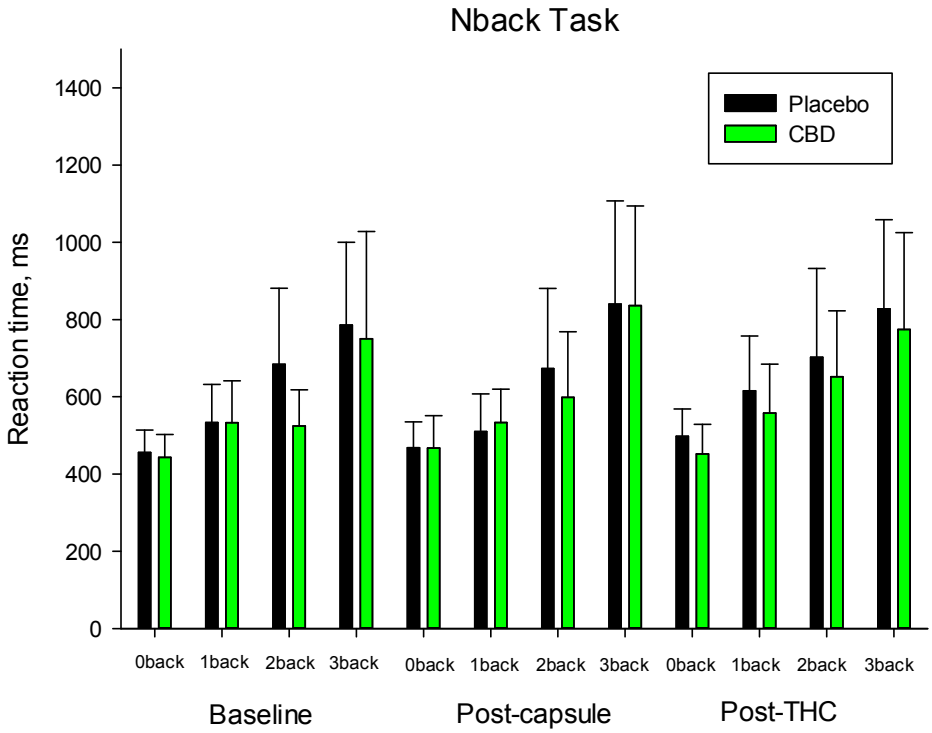


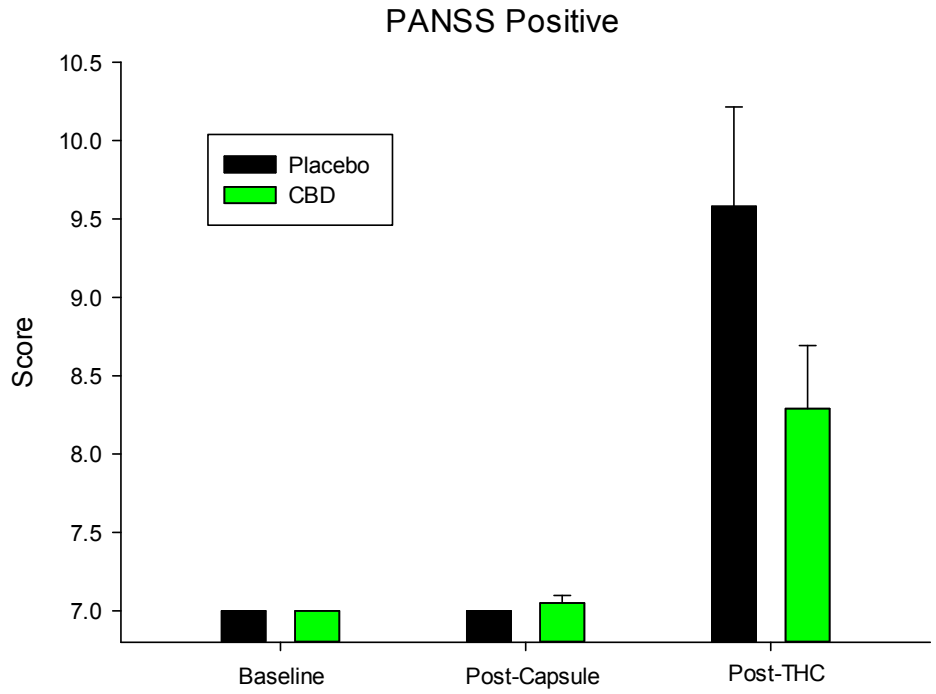
Figure 3.3. Reaction time on the N-back task (mean± SEM)



PANSS

In the placebo group 11 out of 24 participants had a clinically significant psychosis, while only 3 out of 21 in the CBD group reached this threshold (χ^2=5.201, p=0.028). However, when comparing raw PANSS scores both the placebo group (χ^2=26, p<0.001) and the CBD group (χ^2=19.149, p<0.001) showed significant increases across sessions. There was no significant difference between the placebo and the CBD group at the post-Δ9-THC session (Z=-1.225, p=0.221) (Figure 3.4.)

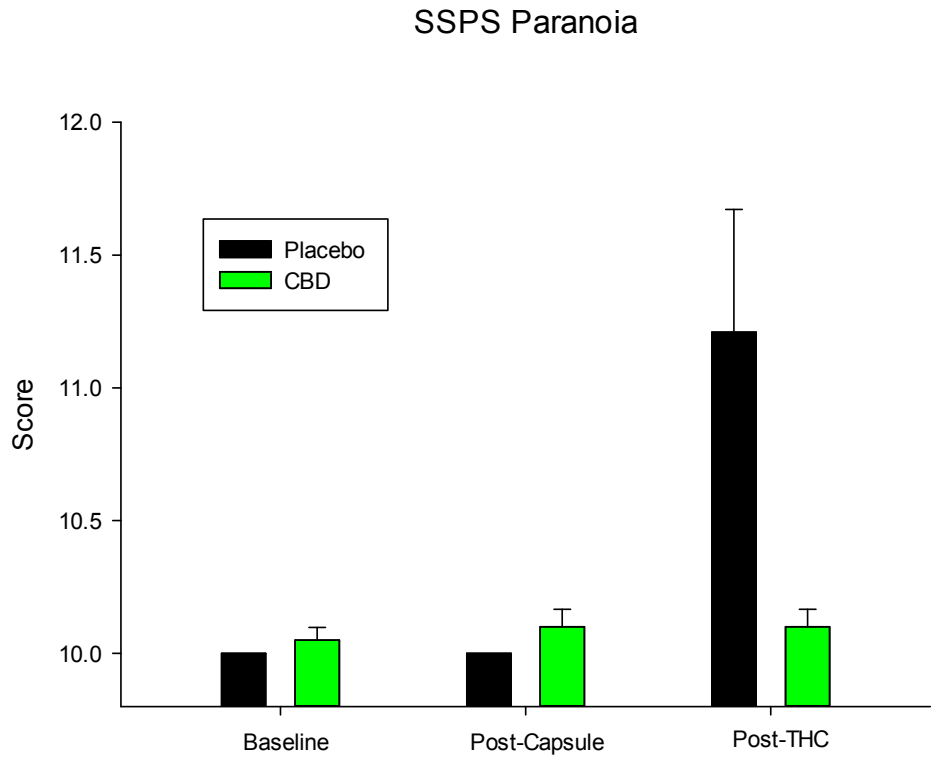
Figure 3.4. Scores on PANSS positive subscale (mean± SEM)



SSPS

There was a significant increase in paranoia scores on the SSPS across sessions in the placebo group (χ^2=16, p<0.001), but not in the CBD group (χ^2=1, p=0.607). There was a significant difference between CBD and placebo groups at the post-Δ9-THC session (Z=-2.096, p=0.036) (Figure 3.5).

Figure 3.5. Scores on SPSS Paranoia (mean± SEM)



Amplitude

Alpha

WM effects

Alpha amplitude significantly decreased with greater difficulty of the n-back task (Load: $F=56.179$, $p<0.001$), where 2-back ($p<0.001$) and 3-back ($p<0.001$) were significantly reduced compared to lower loads. There were significant differences in alpha amplitude with regards to scalp Location ($F=64.017$, $p<0.001$), where the amplitude was greatest at occipio-parietal locations (LOP, ROP, PZ, $p<0.001$) compared to other locations (Figure 3.6).

Δ9-THC and CBD effects

Amplitude significantly increased after each subsequent testing point (Session: $F=18.403$, $p<0.001$), where alpha significantly increased from baseline to post-capsule ($p<0.001$) and from post-capsule to post-Δ9-THC ($p<0.001$). This suggests that alpha amplitude increased as an effect of time rather an effect of Δ9-THC. However, the increased alpha amplitude between post-capsule and post-Δ9-THC was only significant

in the placebo group (p<0.001), while this was non-significant in the CBD group (P=0.651). Δ9-THC significantly influenced alpha increase differently according to scalp location (Location x Session: F=4.462, p=0.001), where Δ9-THC increased alpha increase at frontal and temporal locations while reducing alpha increase at central locations (Figure 3.7). CBD on its own did not influence alpha (F=0.003, p=0.958), and there was no significant Session x Treatment effect (F=1.23, p=0.286) (Figure 3.8).

Figure 3.6. Alpha amplitude according to electrode location (mean± SEM)

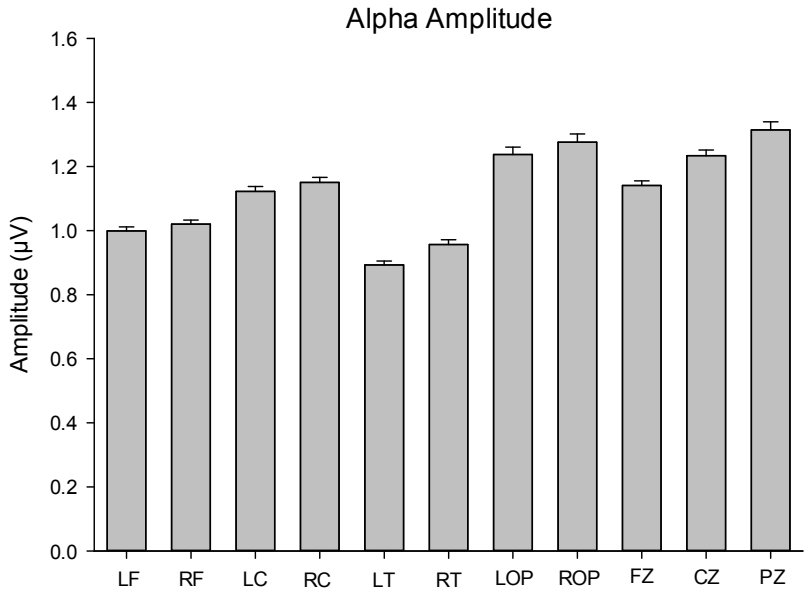


Figure 3.7. Alpha amplitude change across sessions for each scalp location (mean± SEM)

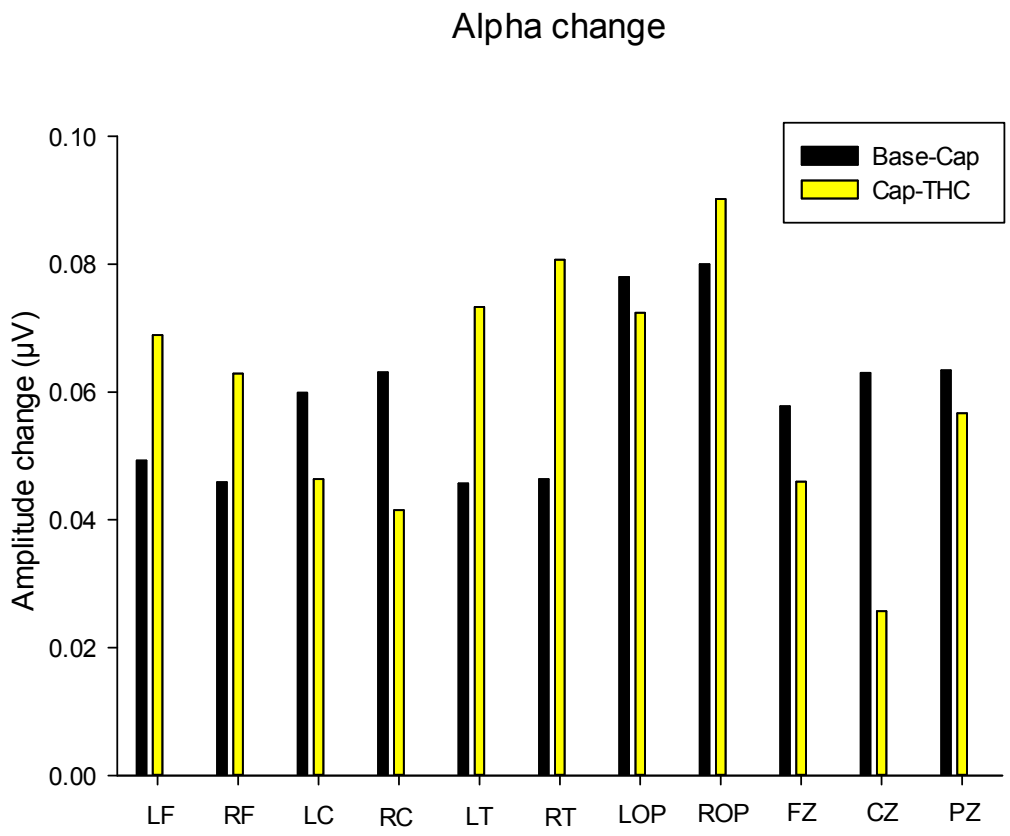
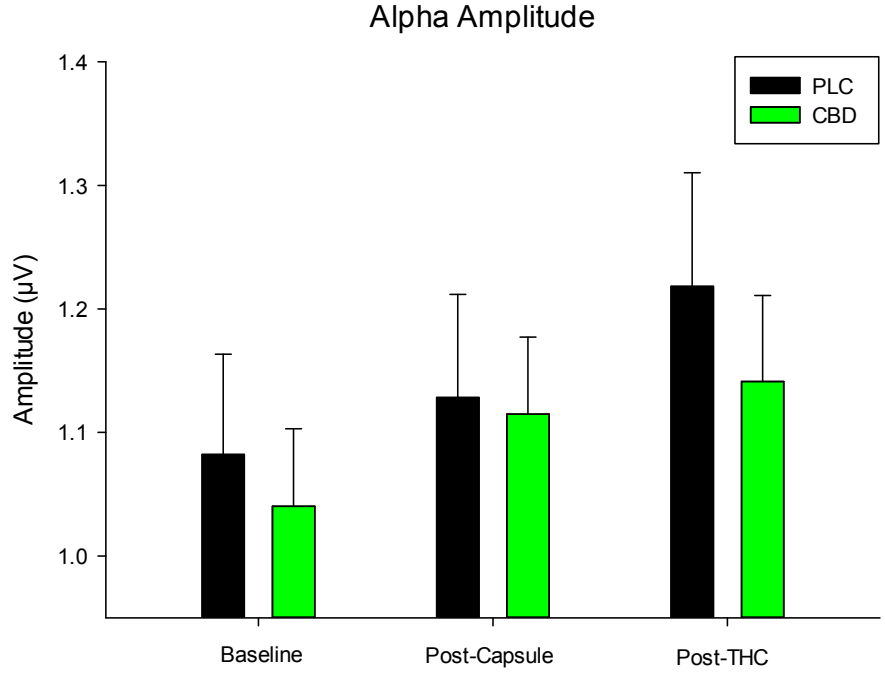


Figure 3.8. Alpha amplitude across sessions for each group (mean± SEM)



Beta

WM effects

There was a significant decrease in beta amplitude as the n-back task became more difficult (Load: $F=88.898$, $p<0.001$), with amplitude being significantly lower during 2-back ($p<0.001$) and 3-back ($p<0.001$) compared to lower loads. Beta amplitude did not significantly differ across scalp locations (Location: $F=1.993$, $p=0.085$) (Figure 3.9).

Δ9-THC and CBD effects

Δ9-THC significantly increased beta amplitude (Session: $F=15.53$, $p<0.001$), and the increase from post-capsule to post-Δ9-THC was significant for both the placebo ($p<0.001$) and CBD group ($p<0.001$). Δ9-THC also affected the influence of WM load on amplitude (Session x Load: $F=2.761$, $p=0.026$), where the difference between 1-back and 2-back was significant at the post-capsule testing point ($p=0.002$) and non-significant post-Δ9-THC ($p=0.184$). Δ9-THC significantly influenced beta increase differently according to scalp location (Location x Session: $F=11.622$, $p<0.001$), where the increase was greatest at temporal locations (Figure 3.10). CBD did not significantly affect beta amplitude (Treatment: $F=0.482$, $p=0.491$), and did not influence Δ9-THC-induced beta amplitude increase (Session x Treatment: $F=1.404$, $p=0.583$) (Figure 3.11).

Figure 3.9. Beta amplitude across electrode location (mean± SEM)

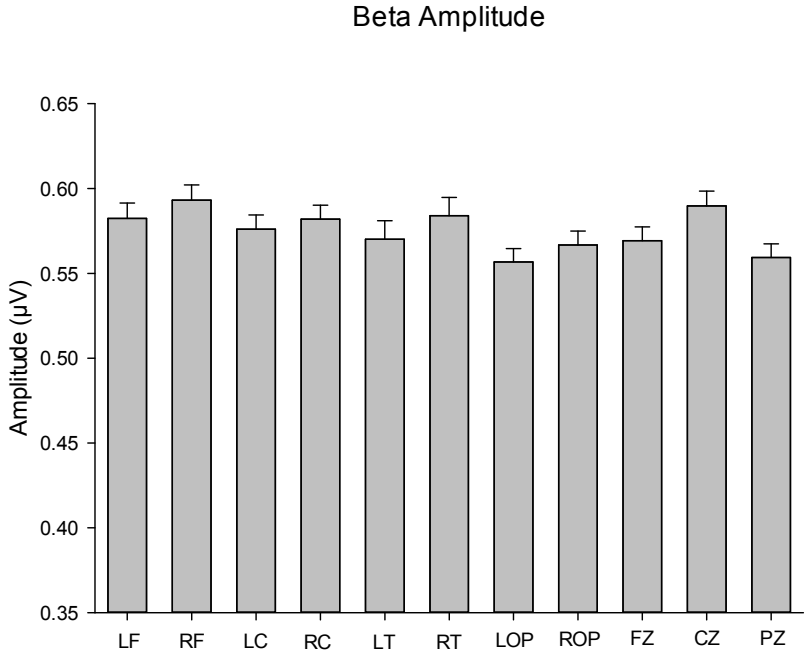


Figure 3.10. Beta amplitude change across sessions for each scalp location (mean± SEM)

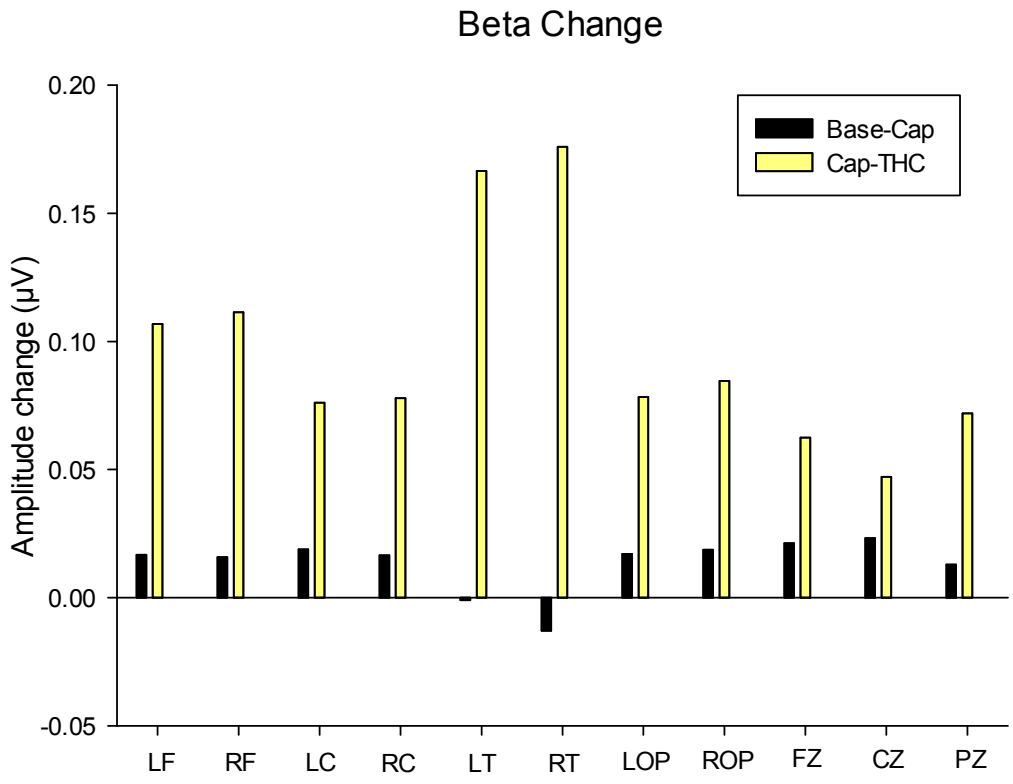
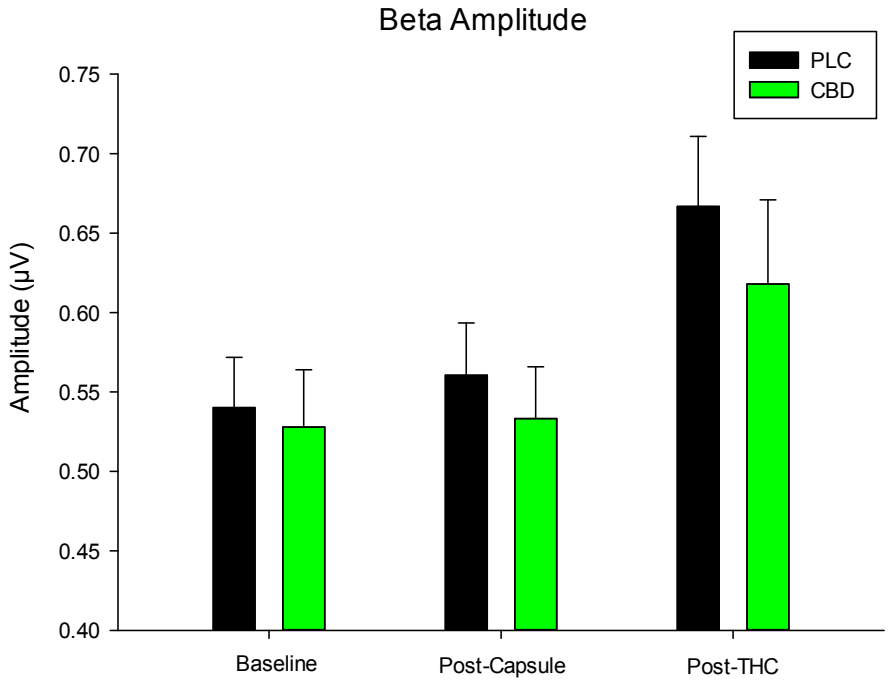


Figure 3.11. Beta amplitude across sessions for each group (mean± SEM)



Delta

WM effects

Delta amplitude significantly decreased with greater difficulty of the n-back task ($F=21.607$, $p<0.001$), 2-back ($p<0.001$) and 3-back ($p<0.001$) were significantly reduced compared to lower loads. There was a significant effect of scalp location on delta amplitude (Location: $F=124.193$, $p<0.001$), where amplitude was significantly greater at FZ ($p<0.001$) and CZ ($p<0.001$) compared to other locations (Figure 3.12).

Δ9-THC and CBD effects

There was a non-significant increase of delta amplitude following Δ9-THC (Session: $F=3.031$, $p=0.077$). Δ9-THC significantly influenced delta amplitude differently according to scalp location (Location x Session: $F=6.073$, $p<0.001$), where Δ9-THC-induced delta increase was greatest at frontal and temporal locations (Figure 3.13). CBD did not influence delta amplitude (Treatment: $F=0.00$, $p=0.991$), although it significantly influenced Δ9-THC-induced delta increase (Session x Treatment: $F=3.787$, $p=0.047$) (Figure 3.14), where delta was increased between post-capsule and post-Δ9-THC in the placebo group ($p<0.001$), but not in the CBD group ($p=0.236$).

Figure 3.12. Delta amplitude across electrode locations (mean± SEM)

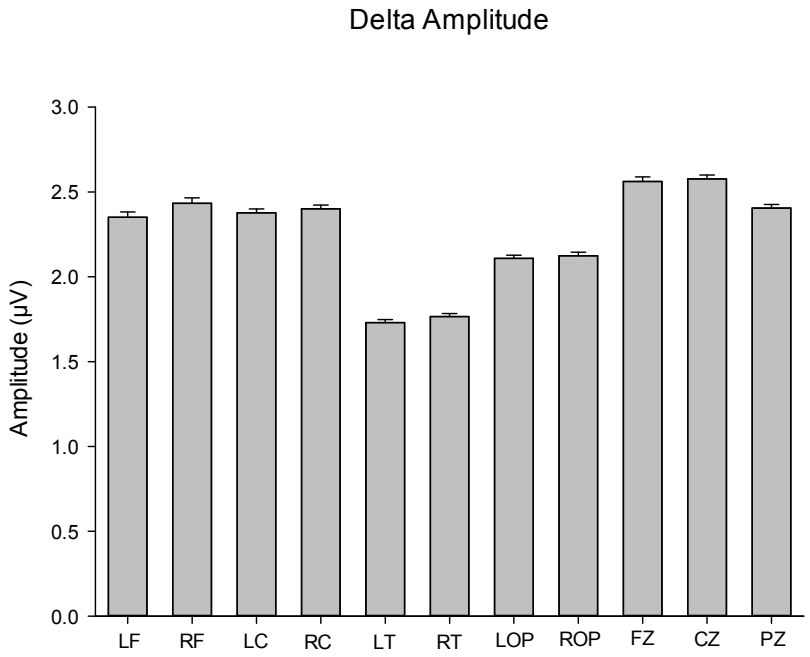


Figure 3.13. Delta amplitude change across sessions for each scalp location (mean± SEM)

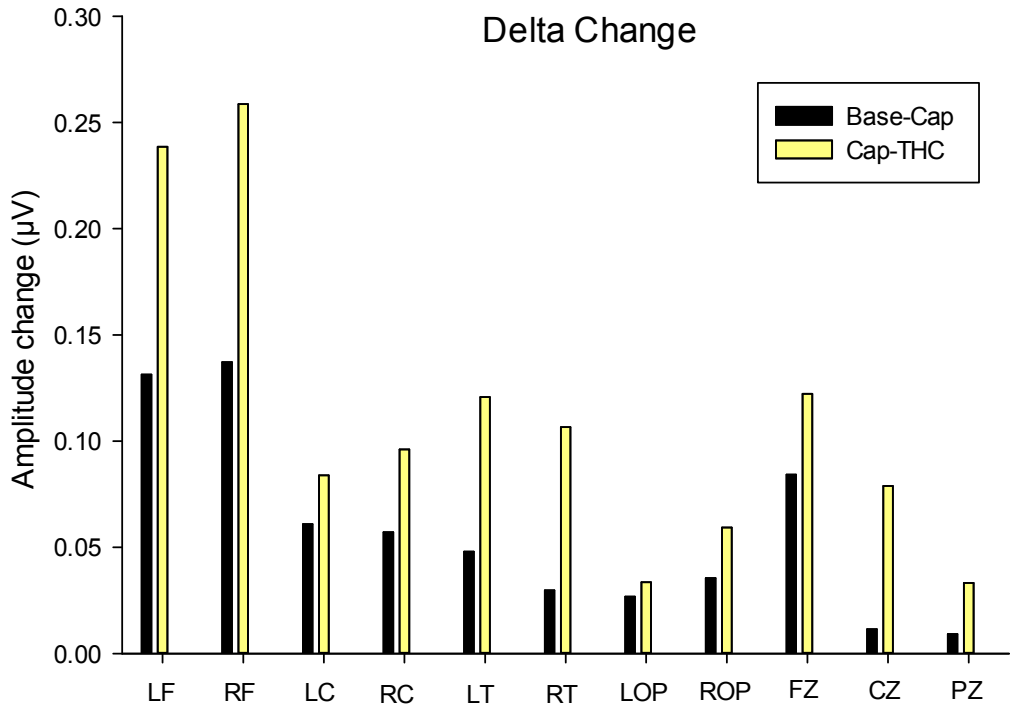
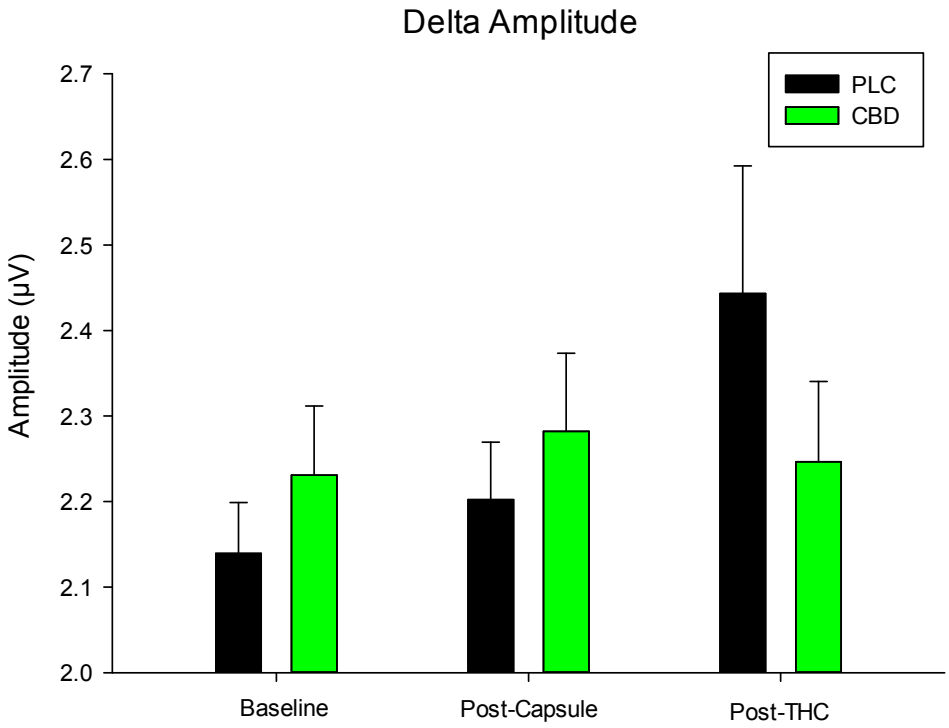


Figure 3.14. Delta amplitude across sessions for each group (mean± SEM)



Theta

WM effects

Theta amplitude significantly decreased with greater difficulty of the n-back task (Load: $F=9.069$, $p<0.001$), although theta was only significantly decreased during 3-back compared to 0-back ($p=0.003$) and 1-back ($p=0.001$). Amplitude significantly differed according to scalp location (Location: $F=250.612$, $p<0.001$), where amplitude was greatest at FZ ($p<0.001$) and CZ ($p<0.001$) compared to other locations (Figure 3.15).

Δ9-THC and CBD effects

There was a significant reduction in theta amplitude following Δ9-THC (Session: $F=15.723$, $p<0.001$) and the decrease from post-capsule to post-Δ9-THC was significant for both the placebo ($p<0.001$) and CBD group ($p<0.001$). Δ9-THC significantly influenced the theta amplitude differently according to scalp location (Location x Session: $F=8.542$, $p<0.001$), where the decrease was greatest at fronto-central locations (Figure 3.16). There was no significant effect of CBD (Treatment: $F=1.18$, $p=0.283$), and CBD did not influence Δ9-THC-induce theta reduction (Session x Treatment: $F=2.475$, $p=0.111$) (Figure 3.17).

Figure 3.15. Theta amplitude across electrode locations (mean± SEM)

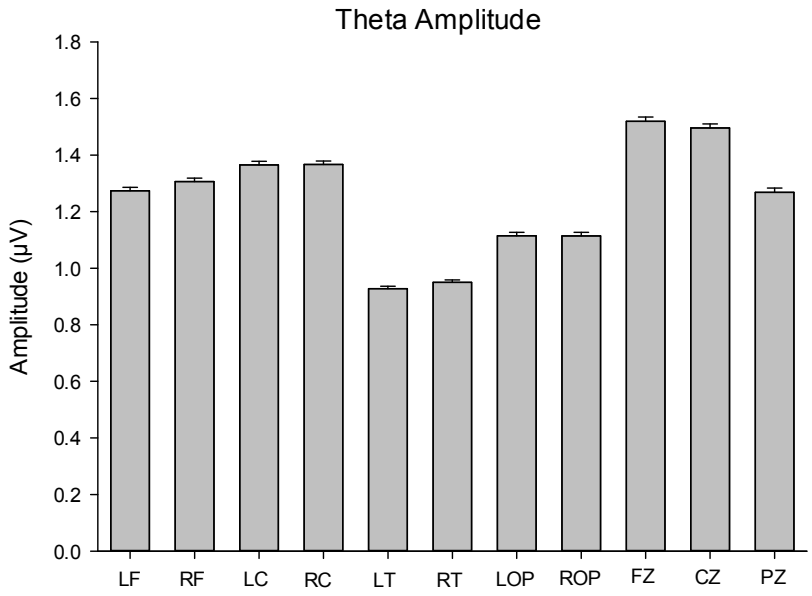


Figure 3.16. Theta amplitude change across sessions for each scalp location (mean± SEM)

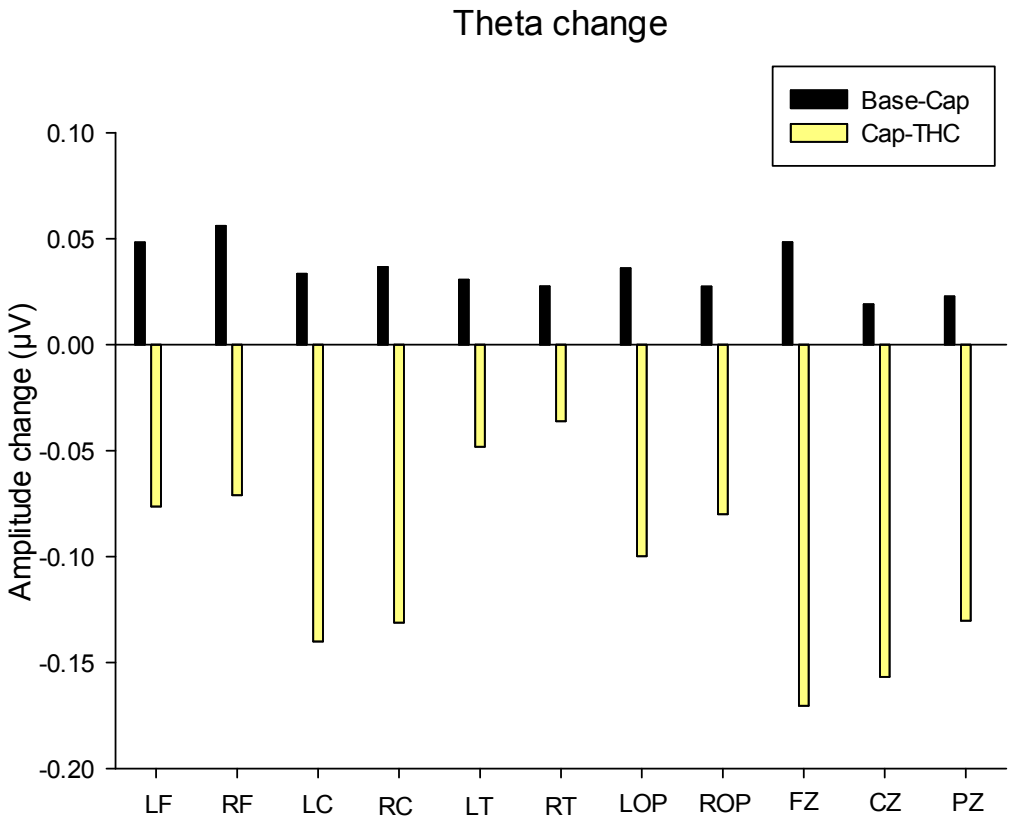
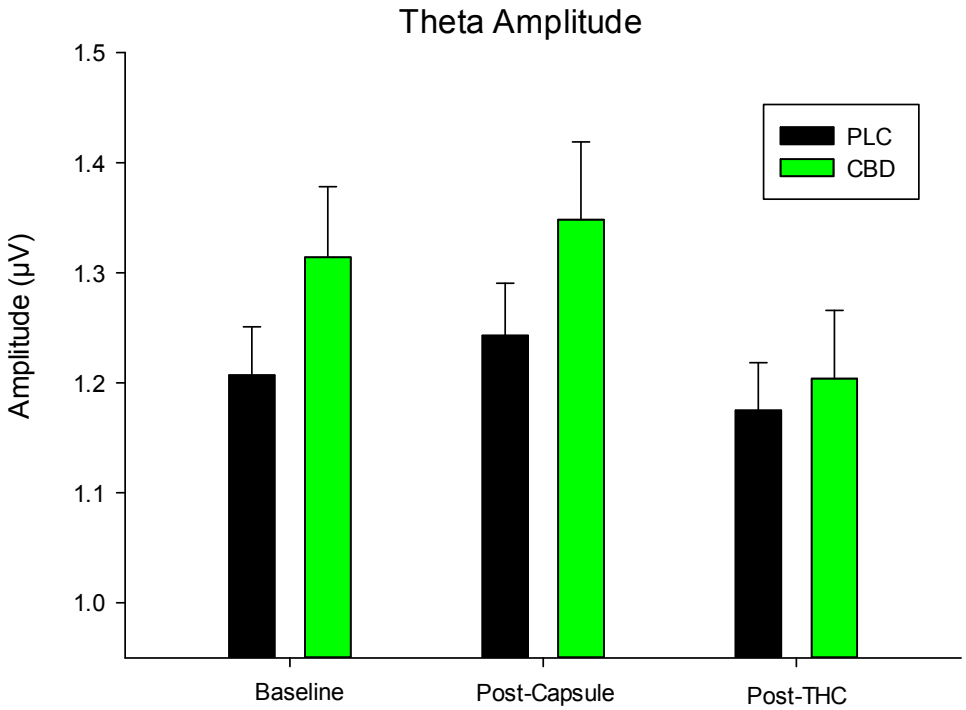


Figure 3.17. Theta amplitude across sessions for each group (mean± SEM)



Gamma

WM effects

Gamma amplitude was significantly decreased by greater working memory load (Load: F=27.635, p<0.001), where 2-back (p<0.001) and 3-back (p<0.001) were significantly reduced compared to lower loads. There was a significant difference in amplitude across scalp locations (Location: F=27.162, p<0.001), where gamma was greatest at frontal and temporal locations (LF, RF, LT, RT: all p<0.05) (Figure 3.18).

Δ9-THC and CBD effects

Δ9-THC significantly increased gamma amplitude (Session: 28.82, p<0.001), where amplitude was significantly increased from post-capsule to post-Δ9-THC in both the placebo (p<0.001), and CBD (p<0.001) group. Δ9-THC significantly influenced gamma amplitude differently according to scalp location (Location x Session: F=1.946, p=0.048), where the increase was greatest at temporal locations (Figure 3.19). There was no significant effect of CBD on gamma (Treatment: F=0.133, p=0.717), and CBD did not influence Δ9-THC-induce gamma increase (Session x Treatment: F=0.271, p=0.710) (Figure 3.20).

Figure 3.18. Gamma amplitude across electrode locations (mean± SEM)

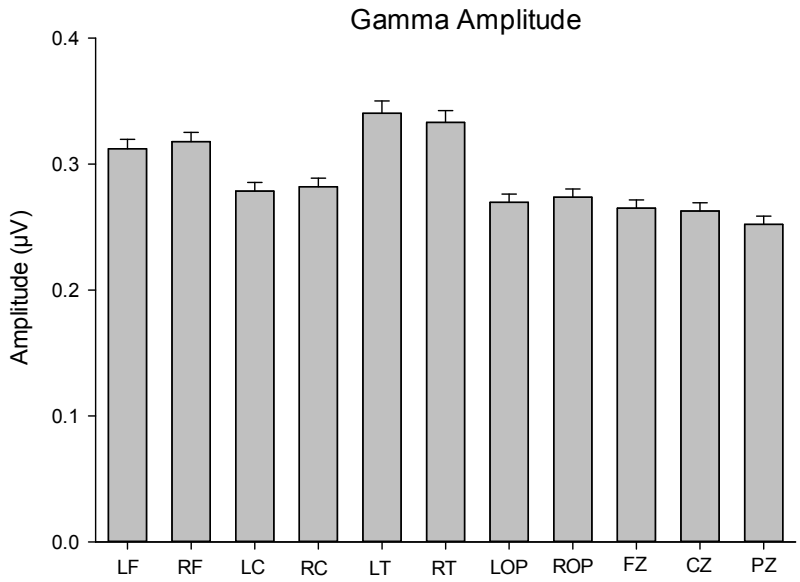


Figure 3.19. Gamma amplitude change across sessions for each scalp location (mean± SEM)

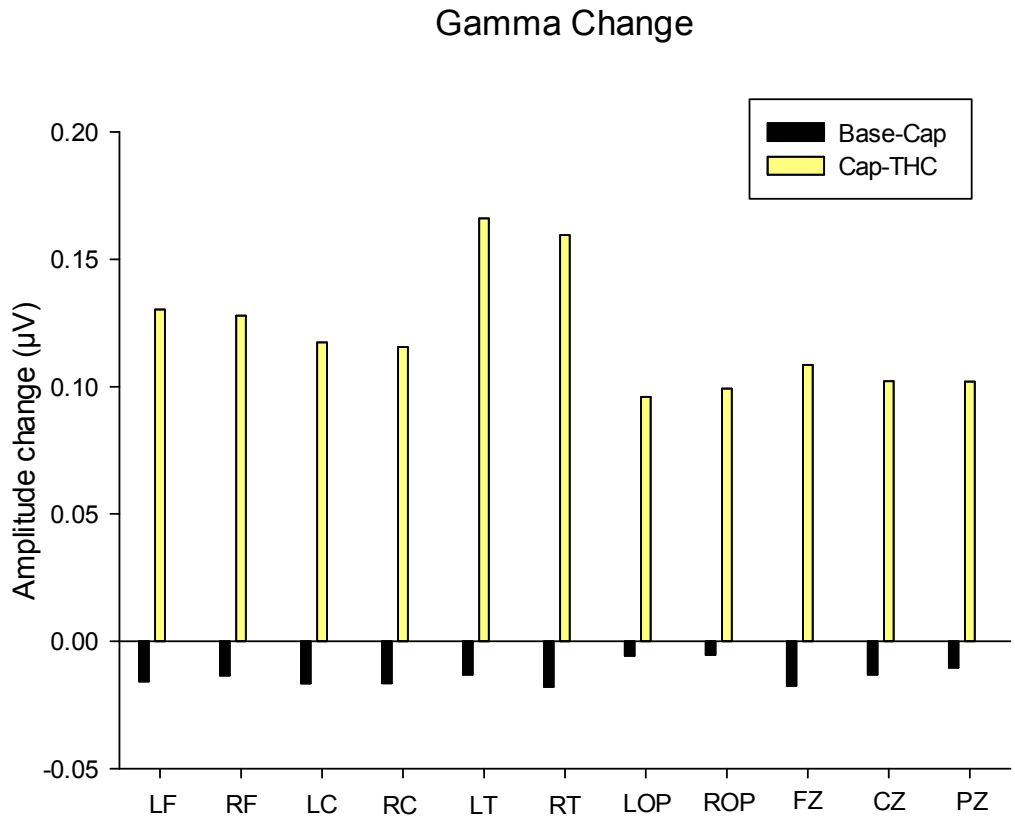
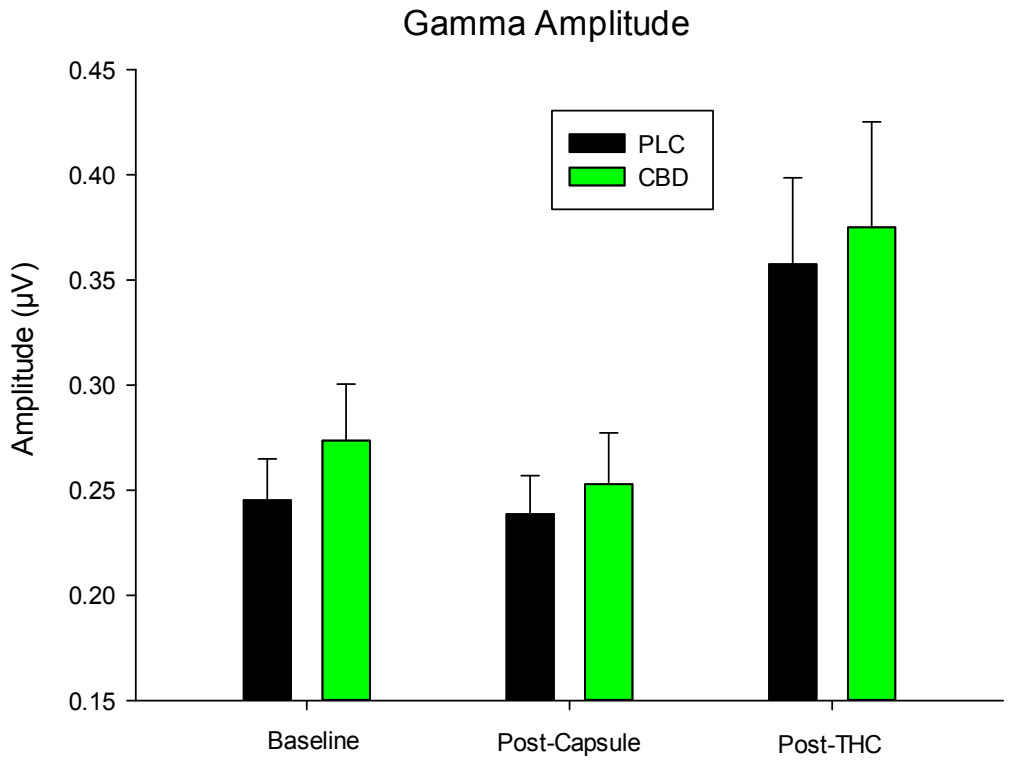


Figure 3.20. Gamma amplitude across sessions for each group (mean± SEM)



Coherence

Alpha

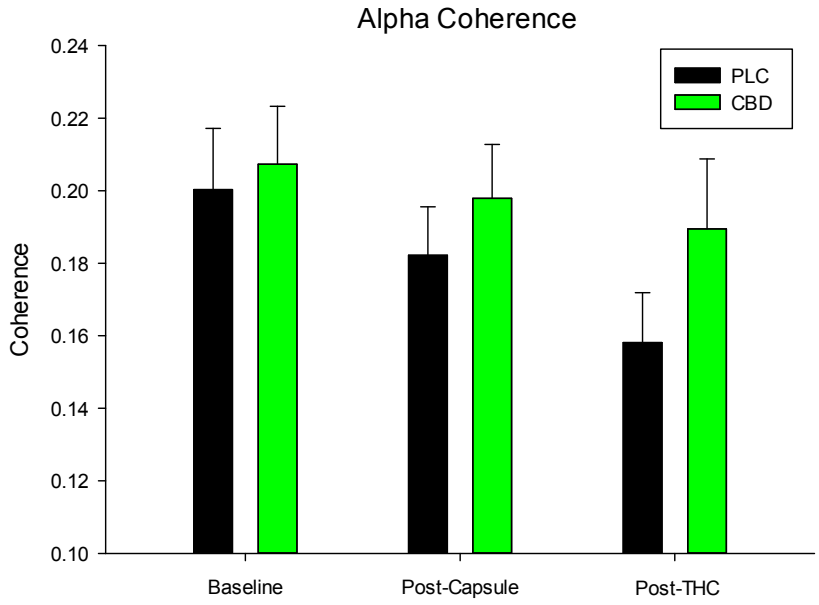
WM effects

Alpha coherence significantly changed across working memory load (Load: F=2.879, p=0.043), although none of the post-hoc comparisons were significant.

Δ9-THC and CBD effects

Bi-frontal alpha coherence was significantly reduced following Δ9-THC (F=3.757, p=0.034), although this reduction was only significant between baseline and post-Δ9-THC (p=0.003) and not between post-capsule and post-Δ9-THC (p=0.221). However, change from baseline to post-Δ9-THC was significant only in the placebo group (p=0.004) but not in the CBD group (p=0.571). CBD did not significantly change alpha coherence (Treatment: F=0.969, p=0.33), and did not influence Δ9-THC-induced reduction of coherence (Session x Treatment: F=0.639, p=0.507) (Figure 3.21).

Figure 3.21. Bi-frontal Alpha coherence across sessions (mean± SEM)



Theta

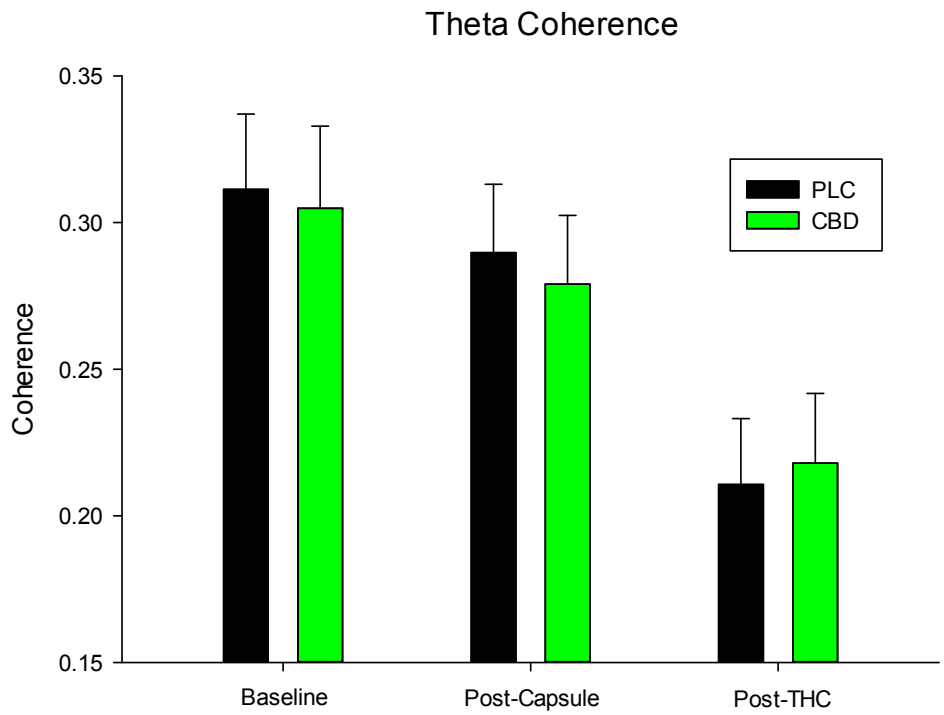
WM effects

Bi-frontal theta coherence significantly increased with greater difficulty on the working memory task (Load: F=16.803, p<0.001), where coherence was reduced for 0-back compared to all other loads (p<0.05) which did not significantly differ from each other.

Δ9-THC and CBD effects

Δ9-THC significantly reduced bi-frontal theta coherence (Session: F=27.986, p<0.001), where coherence was reduced between post-capsule and post-Δ9-THC for both the placebo (p<0.001) and CBD group (p<0.001). CBD did not significantly influence theta coherence (Treatment: F=0.011, p=0.916), and did not influence Δ9-THC-induced reduction in coherence (Session x Treatment: F=0.26, p=0.744) (Figure 3.22).

Figure 3.22. Bi-frontal Theta coherence across sessions (mean± SEM)



Correlations with symptoms

There was no significant correlation between change in EEG coherence and positive psychotic symptoms on the PANSS scale in the theta (rho=0.213, p=0.16) or alpha band (rho=-0.075, p=0.626). Furthermore, increases in paranoid symptoms on the SSPS following Δ9-THC were not correlated with changes to both alpha (rho=-0.116, p=0.331) and theta (rho=0.004, p=0.978) coherence.

Discussion

Psychological effects

Similar to the previous analysis (Englund et al., 2013), exclusion of three participants due to poor EEG data did not significantly change the effects of Δ9-THC on psychopathology. Δ9-THC increased positive psychotic symptoms and paranoia, but this effect was not present in the CBD group, suggesting a protective effect of CBD.

Cognitive effects

Although cognitive performance was impaired on certain tasks in the previous analysis, there was only a trend towards impaired performance or reduced reaction time on the n-back task following Δ9-THC. Furthermore, CBD did not influence performance on the n-back task before or after Δ9-THC.

Theta

The main frequency band of interest for this study was theta, as previously shown to be significantly affected by Δ9-THC (Reich et al., 2005; Hajós et al., 2008; Kucewicz et al., 2011; Morrison et al., 2011; Robbe et al., 2006; Ilan et al., 2004, 2005; Koen B E Böcker et al., 2010). Particularly Morrison and colleagues who also used intravenous Δ9-THC, showed significant decreases in theta power and coherence, the latter being correlated to positive psychotic symptoms (Morrison et al., 2011). In this study I have replicated these findings where both theta amplitude and bi-frontal theta coherence was significantly reduced following Δ9-THC.

I was however unable to find a significant relationship between change in theta coherence and increase of positive psychotic symptoms. Interestingly, although CBD significantly reduced symptoms of psychosis and paranoia, it did not inhibit Δ9-THC-induced theta amplitude and coherence reductions. The observation here that theta amplitude and coherence were reduced while being unaffected by CBD may suggest that theta may not be related to Δ9-THC-induced psychotic symptoms. It has been previously demonstrated that theta is related to increased focus and concentration (Gevins et al., 1997), which may provide a better explanation for the theta reductions in this study. Previous studies have consistently found an association between Δ9-THC-induced reduced theta and impaired performance on cognitive tasks (Ilan et al., 2004; Koen B E Böcker et al., 2010; Kucewicz et al., 2011). However, in this study there was only a trend towards poorer accuracy on the n-back task following Δ9-THC, which may be explained by a practice effect since the participants had performed the n-back task twice before during the same day. Theta reduction may therefore reflect intoxication in this instance. Furthermore, CBD alone had no influence on either amplitude or coherence, which may suggest that it does not significantly impact normal network oscillations.

Alpha

There was a significant increase in alpha amplitude across sessions, where the increase from baseline to post-capsule was comparable to post-capsule and post-Δ9-THC. This may suggest an increase as an effect of participants becoming more fatigued as the experimental day went on. There was however no increase of alpha in the CBD group following Δ9-THC which may be explained as a synergistic effect of Δ9-THC and CBD. Juckel and colleagues found that MMN was significantly increased by cannabis extract containing both Δ9-THC and CBD, while there was no change under Δ9-THC alone (Juckel et al., 2007). They argued that this might indicate a beneficial effect of the combination of Δ9-THC and CBD as reduced MMN has been previously been associated with schizophrenia (Javitt et al., 1995). This would not seem to be the case in this study as there was still an increase of positive psychotic symptoms in the CBD group following Δ9-THC, albeit less than in the placebo group.

Alpha rhythms are commonly seen in the occipital cortex when the participant's eyes are closed, which is said to reflect cortical networks in "stand-by" (Compston, 2010). More recently it has been argued that alpha may be viewed as representing functional inhibition of processes which are not related to the task at hand (Sauseng et al., 2007). This would seem to fit in with the results from the current study as alpha amplitude decreased the more participant's had to focus on the task (increased WM load), as well as alpha increasing for each subsequent testing session (participant fatigue).

Bi-frontal alpha coherence was significantly reduced following Δ9-THC, an effect which was absent in the CBD pre-treated group. Coherence in the alpha band has been related to long-distance communication between cortical regions (von Stein et al., 2000). A reduction in coherence following Δ9-THC may therefore reflect reduced communication between frontal regions due to Δ9-THC, resulting in impairments to perception and cognition. Similar to amplitude, alpha coherence was not reduced following Δ9-THC in the CBD group, which may suggest a protective effect of CBD. However, this seems unlikely as alpha coherence was not correlated with positive symptoms, as previously demonstrated in a similar study with intravenous Δ9-THC (Morrison et al., 2011). Similarly to theta, CBD did not influence alpha amplitude or coherence.

Delta

There was no significant effect of CBD alone on delta amplitude. However, amplitude was significantly increased by Δ9-THC, and this increase was more pronounced in frontal and temporal regions compared to other locations. Similar to what was seen in alpha, the increase from post-capsule to post-Δ9-THC was not observed for the CBD group, which suggests that CBD has an inhibitory effect on Δ9-THC-induced delta increase. Delta activity is a main component of slow wave sleep, although increases in delta during waking states are commonly found in intoxicated, confused and disease states. Increased delta power while awake has been observed in delirium (Jacobson and Jerrier, 2000), as well as among patients with schizophrenia and their relatives (Alfimova and Uvarova, 2007). In this study, I find it unlikely that the Δ9-THC-induced delta increase is related to delirium or intoxication as CBD did not affect the intoxicating effects of Δ9-THC. As CBD inhibited Δ9-THC-induced delta increase as well

as symptoms of psychopathology, it may be argued that the increase in delta observed is related to paranoid or psychotic symptoms. However, these findings should be interpreted with caution as previous studies with Δ9-THC in humans have either found no change in delta (Koen B E Böcker et al., 2010) or a reduction (Morrison et al., 2011). Furthermore, increased delta (particularly frontally) may also be an eye movement artefact, which might suggest that Δ9-THC increased eye movements.

Beta

There was a significant increase in beta amplitude following Δ9-THC which was seen in both the placebo and CBD group. Δ9-THC also increased beta amplitude differently according to scalp location, where the increase was greatest at temporal regions. The finding that beta was increased by Δ9-THC is contrary to previous Δ9-THC studies where generally Δ9-THC has been found to reduce beta (Ilan et al., 2004, 2005; Hart et al., 2010; Morrison et al., 2011). However, Böcker and colleagues observed an increase in beta for the doses of 29mg and 49mg, while the 69mg dose produced a drop in beta power (Koen B E Böcker et al., 2010). Beta activity is commonly considered to be related to states of alertness, activity and possibly even anxiety. One study found that decreased beta activity was related to feeling more relaxed (Baumeister et al., 2008). The disparity between the results of this study with past findings may be explained by the higher dose and intravenous administration of Δ9-THC which might have made participants feel more anxious or alert compared to previous studies. Furthermore, CBD did not change beta amplitude or affect Δ9-THC-induced beta increase. An alternative explanation may also be that Δ9-THC made participants clench their jaws more and increasing beta amplitude, particularly since the greatest increase was seen in temporal locations.

Gamma

Similar to beta, gamma amplitude was also significantly increased following Δ9-THC, with no protective effects of CBD. This is in line with previous intravenous Δ9-THC studies in humans (Morrison et al., 2011), although animal studies have consistently shown reductions to gamma in the hippocampus (Hajós et al., 2008; Hájos et al., 2000; Kucewicz et al., 2011). These discrepancies may be explained by the use of the more

potent CB1 agonists CP55940 which is roughly 45 times as potent as Δ9-THC (Rinaldi-Carmona et al., 1996), but also that the recoding electrodes were implanted directly onto the hippocampus as opposed to mere scalp recordings. Furthermore, scalp gamma recordings are vulnerable to a significant amount of artefacts from tonic muscle activity, which can be minimised using modern artefact reduction techniques (Nottage et al., 2013). Since the main focus of this study is the effects of cannabinoids on the lower frequency bands such artefact reductions were not performed, which limits the reliability of the findings. Also, similar to beta amplitude gamma may also have increased as a result of increased muscle tension of the jaw.

Mechanisms

Neural oscillations originate from many different locations depending on frequency, including the thalamus, hippocampus, basal ganglia, entorhinal, prefrontal, somatosensory, motor, and visual cortex (Uhlhaas et al., 2008). Most of these regions come under the control of glutamatergic or GABAergic neurons which regulate oscillations, and are also highly innervated with cannabinoid receptors (Howlett et al., 2002). Disruptions to the finely tuned "on-demand" activity of the endocannabinoid system by means of exogenous cannabinoid agonists may therefore result in over-excitation by glutamate or over-inhibition by GABA. The disrupted, or lack of, regulatory input from the endocannabinoid system leads to significant changes to normal network oscillations. This is exemplified by a study on CB1-knockout mice where several alterations to EEG activity during were observed compared to wild-type mice (Silvani et al., 2014).

The disruptive effects of Δ9-THC on EEG activity in this study are likely due to over-stimulation of CB1 receptors. This over-stimulation resulted in decreased theta amplitude while all other frequency bands increased, also there were significant decreases in theta and alpha coherence. In the case of alpha and delta amplitude, CBD significantly protected against the amplitude increase. It may therefore suggest that CBD acts specifically within these frequency bands while having no impact on others.

CBD alone did not influence any EEG measure in this study. This is in line with studies suggesting CBD acts as an endocannabinoid enhancer, by inhibiting the breakdown or reuptake of endocannabinoids (Stern et al., 2012; Bitencourt et al., 2008; Leweke et

al., 2012; Capasso et al., 2008). Since the endocannabinoid system does not produce and release endocannabinoids until post-synaptic calcium levels have increased ("on-demand"), increased endocannabinoid levels from CBD would not have an effect until the system came under some form of (neural) stress. CBD has been previously shown to reduce stress in healthy human volunteers (Crippa et al., 2011; Bergamaschi et al., 2011), while producing no effects in healthy volunteers without stress (Hollister, 1974; Perez-Reyes et al., 1973). In this study only healthy volunteers were recruited, hence this may be a population for which CBD has little or no effect on psychological and electrophysiological measures.

Strengths and limitations

This study benefitted from administering Δ9-THC intravenously as opposed to oral or inhaled which significantly reduces inter-individual variation in bioavailability. Furthermore, only pure synthetic drugs were used which allows conclusions regarding specific pharmacological effects of Δ9-THC and CBD to be drawn. Cannabis extracts of smoked cannabis contain a large variety of cannabinoids (with varying concentrations of each) and other pharmacologically active components such as terpenoids and flavonoids (Russo and McPartland, 2003).

Due to the high variation in clinical responses to Δ9-THC among healthy volunteers (D'Souza et al., 2004; Morrison et al., 2009; Englund et al., 2013), this study may have been improved upon by employing a repeated measures design. This would have allowed each participant to act as their own control and therefore could have improved the power of the study. Furthermore, as the experimental day was very long (starting ~9am and finishing ~5pm) and contained repeated sessions of cognitive tasks, it is likely this impacted on some of the EEG measurements. This may be potentially relevant as participant fatigue could have influenced some of the EEG changes seen following Δ9-THC. However, increased theta and decreased alpha were observed during working memory which is a highly robust EEG finding for WM tasks (McEvoy et al., 2000; Gevins and Smith, 2000; Gevins et al., 1998; Ilan et al., 2004). Furthermore, some baseline differences in EEG measures were observed between the two groups which may have influenced the results of the analysis. However, post hoc analyses revealed no significant differences between baseline measures.

Future studies would benefit from employing a repeated measures design to better capture the variation in responses to a set dose of Δ9-THC to confirm the findings of the current study. Exploring a wider dose-range for both Δ9-THC and CBD would also highlight potential dose response effects on measures of cognition, psychology, and EEG. Lastly, the length of the experimental sessions should be taken into consideration as long testing days with many and demanding cognitive and psychological tasks may confound some of the EEG measures.

Conclusion

The main findings of this study are that Δ9-THC decreases both theta amplitude and coherence, which is in line with previous studies on EEG effects of cannabinoids, although an association between these changes and psychopathology was not found. Alpha and delta amplitude were significantly increased, but this effect was absent in the CBD group which suggests a protective effect against these Δ9-THC-induced changes. CBD alone did not influence any EEG measure in this study, which may indicate that CBD is more active against a disrupted or diseased network.

Cognitive and psychological effects of Δ9-THC and Δ9-THCV

Introduction

The effects of cannabis are highly dependent upon the various amounts of the different active components of the plant, the cannabinoids (Englund et al., 2013; Morgan, Schafer, et al., 2010; Schubart et al., 2011; Zuardi et al., 1982). The Cannabis Sativa L. plant produces over 80 different cannabinoids (Izzo et al., 2009). Each cannabinoid is produced in various concentrations depending mostly on the specific genetic makeup of the individual strain, but also factors such as degrees of lighting, temperature and nutrition (Potter, 2013). The main active component of cannabis is Δ9-tetrahydrocannabinol (Δ9-THC) which is the most abundant cannabinoid produced by the plant, while the second most common is cannabidiol (CBD) (Potter, 2013). A lesser common cannabinoid is Δ9-tetrahydrocannabivarin (Δ9-THCV), which often exists in very low quantities in most cannabis varieties (Mehmedic et al., 2010). However, certain strains of cannabis have been identified which are particularly rich in Δ9-THCV (Hillig and Mahlberg, 2004).

Hollister and colleagues were the first to administer pure Δ9-THCV to six healthy volunteers. The participants received an intravenous dose of 7mg Δ9-THCV (Hollister, 1974). One of the participants noticed no effects while the others reported mild to moderate cannabis-like effects. The authors concluded that Δ9-THCV is roughly 25% as psychoactive as Δ9-THC. Δ9-THCV was initially thought to be a weak agonist at the CB1 receptor as previous animal studies had indicated (Gill et al., 1970). A more recent study highlighted this by demonstrating that the antinociception produced by Δ9-THCV was inhibited by the known CB1 receptor inverse agonist SR141716A (Rimonabant) (Pertwee et al., 2007). Although it seems as if Δ9-THCV functions as a CB1 agonist, there is also evidence that it acts as a CB1 and CB2 antagonist at lower doses (Thomas et al., 2005). Furthermore, Δ9-THCV has been shown to inhibit the effects of Δ9-THC in mice (Pertwee et al., 2007), while not exhibiting characteristics of a CB1 inverse agonist (Rock et al., 2013). This had led researchers to conclude that Δ9-THCV is a CB1 receptor neutral antagonist (Wargent et al., 2013). A neutral antagonist is a compound which binds to a receptor but has 0% efficacy as opposed to an agonist which has at

least some efficacy or an inverse agonist which lowers the baseline cellular activity. A recent systematic review strengthened this notion as it found that 3 out of 4 efficacy studies were consistent with THCV as a neutral antagonist, while the fourth found it to be more similar to an inverse agonist (McPartland et al., 2014). Although the precise pharmacodynamic profile of THCV has not yet been fully elucidated it appears most likely that it acts as a neutral antagonist in the lower dose-ranges, while possibly acting as an agonist at higher doses. However, the notion of THCV as an agonist at higher doses needs replication as it is merely based on a small sample.

There remains worry regarding blockade of CB1 receptors in the central nervous system as this has been linked to unfavourable psychiatric side effects. Rimonabant, which is a CB1 receptor full inverse agonist was marketed as an anti-obesity drug and showed very promising results. A meta-analysis found that Rimonabant significantly reduced weight (mean 4.7kg over 12months), waist circumference and improved cholesterol values (Christensen et al., 2007). However, due to concerns regarding significant increase of anxiety, depression and suicidal ideation in the patients treated with Rimonabant, the drug was withdrawn from the market (Nissen et al., 2008). More recently, studies with Rimonabant in healthy volunteers has shown it to produce a bias towards remembering negatively loaded words (Horder et al., 2012) and increasing anxiety during a public speaking task (Bergamaschi et al., 2014). CB1 antagonists have also shown some indication of improving memory functioning in animals (Shiflett et al., 2004; Terranova et al., 1996; Lichtman, 2000), although this has not yet been observed in humans (Horder et al., 2009; Boggs et al., 2012).

It has been previously demonstrated that an acute administration of a high dose Δ9-THC can provoke schizophrenia like psychotic symptoms and cognitive impairment in roughly 40-50% of healthy volunteers (D'Souza et al., 2004; Morrison et al., 2009). This is particularly relevant as Δ9-THC levels in cannabis sold on the illegal market are on the rise (Mehmedic et al., 2010), and globally have roughly doubled since the 1970s, with large variation between countries (Cascini et al., 2012). Furthermore, levels of CBD, which have been shown to protect against the effects of Δ9-THC (Englund et al., 2013; Schubart et al., 2011), have either remained low or declining (Potter et al., 2008; Mehmedic et al., 2010).

Recent studies have highlighted that frequent use of cannabis products high in Δ9-THC and low in CBD pose a significantly greater risk of psychosis (Di Forti et al., 2009), as well as an earlier onset of the illness (Di Forti et al., 2013). Morgan and colleagues also showed that stronger strains of cannabis with less CBD have a greater negative impact on memory function (Morgan, Schafer, et al., 2010). In this small pilot study, I report the findings of 5 days dosing with Δ9-THCV followed by intravenous administration of Δ9-THC in 10 healthy male volunteers. I hypothesise that Δ9-THCV will inhibit Δ9-THC induced psychotic symptoms, paranoia and cognitive impairment.

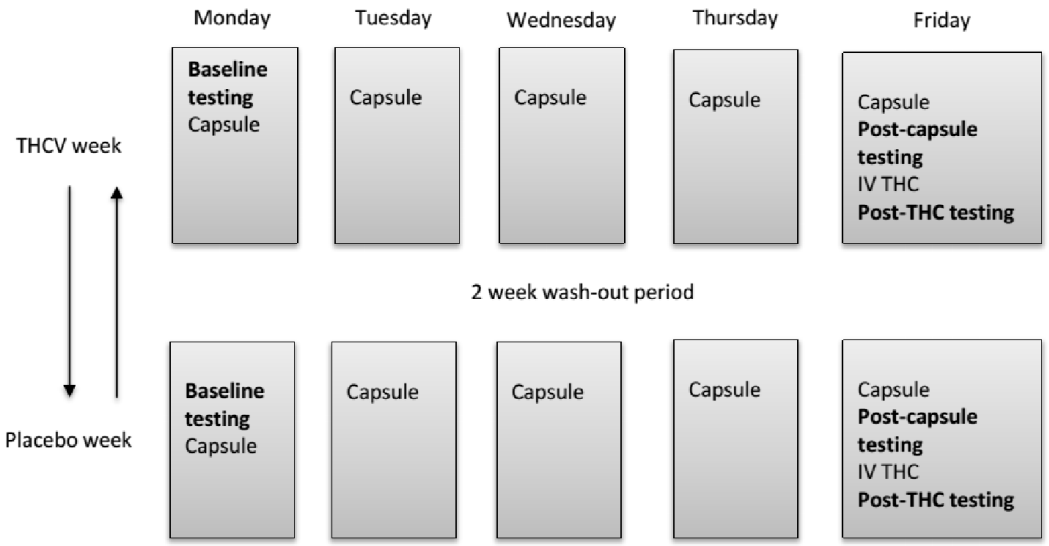
Methods

The study was approved by the Camden & Islington National Research Ethics Committee. All subjects were given time to study the participation sheet and provided written informed consent (appendix 1). The safety of intravenous Δ9-THC administrations has been previously reviewed (Carbuto et al., 2012). Participants were informed of the possibility of short-lived anxious and psychotic like effects of intravenous Δ9-THC, and were made aware of stopping procedures of the study as well as the possibility of receiving rescue medication (Lorazepam 1-4mg).

Design

This was a randomized, double-blind, placebo-controlled, cross-over study in which participants were dosed for 5 days with Δ9-THCV or placebo before administration of intravenous Δ9-THC. A minimum of two weeks wash out period was allowed between each testing week. Consent and screening of participants were carried out on a separate occasion prior to the first testing week. The testing weeks consisted of baseline assessment on a Monday, which followed administration of either Δ9-THCV or placebo. Participants then returned on Tuesday, Wednesday and Thursday for additional dosing and monitoring of side-effects. The Friday session started with administration of the final dose of Δ9-THCV or Placebo, followed by Post-Capsule testing, IV Δ9-THC administration and Post-Δ9-THC testing (see Figure 4.1). All participants provided a clean urine drugs screen at the start of each testing week, but not at the day of the experiment as this would test positive to metabolites of Δ9-THCV and unblind the researcher. Vital signs were tested on every visit.

Figure 4.1. The timeline of the experimental weeks. The order of assignment to placebo or Δ9-THCV treatment was randomised.



Participants

Ten healthy male volunteers were recruited for this pilot study by means of recruitment emails sent to staff and students of King's College London. Participants aged 21-35, with a minimum lifetime cannabis use of at least once and no more than 25 times were invited for screening and consent. The reason for this narrow inclusion criteria of previous cannabis exposure was to try and minimise variation in our sample as previous studies have found that frequency of use can affect reactions to THC (Hart et al., 2010; D'Souza, Ranganathan, et al., 2008). As funds were limited for this study we wanted to keep the sample as homogenous as possible in terms of gender, age and past use of cannabis. Exclusion criterion included a history of mental illness (psychotic disorder, depression, anxiety), major mental illness in first degree family member (e.g. schizophrenia), major physical illness, previous treatment with psychotropic medications, past or present drug and alcohol dependence (excluding nicotine) and being unable or unwilling to give written informed consent. All participants provided written informed consent. Participant demographics are presented in Table 4.1.

Table 4.1. Participant demographics

No. participants	10
Age (Mean (range))	23.8 (21-33)
Handedness	2 left handed
BMI (mean (range))	22.69 (18-27.4)
Education	4 College degree - 6 University degree
Cannabis	
Use status	7 past users
Age of first use (mean (range))	18.6 (16-27)
Lifetime us 12.9 (3-25)	
Other drug exposure	No. of participants
Tobacco	8
Alcohol (m 10 (9, 2-20)	
Amphetamine	1
LSD	3
Ketamine	2
Psilosibin	2
MDMA	4
Mephedrone	2
Cocaine	4
Nitrus oxide	1
2-CB	1

Pharmaceuticals and Pharmacokinetics

Δ9-THCV (2 x 5mg capsules) and matching placebo were provided by GW Pharmaceuticals UK. Synthetic Δ9-THC was acquired from STI Pharmaceuticals UK, via Δ9-THC Pharm GmbH (Frankfurt am Main, Germany) and prepared as 1 mg/mL vials for IV injection, by Bichsel Laboratories (Interlaken, Switzerland). Oral Δ9-THCV (10mg) was administered each day for 5 days to reach steady state plasma concentration of Δ9-THCV. This was based on recommendations and limited data from GW Pharmaceuticals. Intravenous Δ9-THC was prepared as a solution containing 9ml normal saline and 1ml Δ9-THC. Δ9-THC was administered over 10 minutes with 1ml/min pulses (total dose 1mg). For this study we chose a slightly lower dose of IV Δ9-THC compared to our previous studies (Englund et al., 2013; Morrison et al., 2011; Stone et al., 2012; Morrison et al., 2009), to better highlight the possible inhibitory

effects of Δ9-THCV in case they are easily overpowered by a high Δ9-THC dose. Blood samples to measure plasma cannabinoid levels were taken prior to the administration of the final oral tablet (baseline), followed by 5min, 15min, and 1h after the end of intravenous Δ9-THC infusion. Plasma cannabinoid levels were analysed at Quotient Bioresearch (Cambridgeshire UK), where ultra performance liquid chromatography – tandem mass spectrometry was used for quantification of Δ9-THC, Δ9-THCV, 11-OH-Δ9-THC and 11-OH-Δ9-THCV. The lower limit of quantification was 0.25 ng/mg and the upper limit of quantification was 250 ng/ml.

Cognitive tasks

The Hopkins Verbal Learning Task-Revised

The Hopkins Verbal Learning Task – Revised (HVLT) is a part of the MATRICS Consensus Cognitive Battery (PAR, Inc FL 33549). It consists of learning a list of 12 words across three trials (nouns from three taxonomic categories), followed by recall 20-25 minutes later, of which there are five validated versions. The versions were randomised between each participant and version 1 was used for both baseline testing points. The HVLT tests the participants' performance on immediate and relayed recall, as well as taking note of intrusions and repetitions. Immediate recall is measured as the total number of words recalled during the three learning trials. Delayed recall is measured as the percentage of correctly recalled words compared to the best trial from the learning phase. Repetitions refer to number of times a correctly recalled word is repeated, and intrusions are words recalled that are related to the words in the list yet not part of the original list.

Digit Span

The Digit Span refers to the longest list of numbers the participant can correctly recall, both in forward and backward order. The task starts at the length of 4 digits and is increased by one digit for each successful trial. The task is ended when the participant fails to give the correct order after two consecutive attempts.

Psychological scales

Community Assessment of Psychic Experiences-state (CAPE-state)

The CAPE-state is a 42-item validated scale which measures positive, negative and depressive dimensions of psychotic-like experiences (Stefanis et al., 2002), where each item has a yes/no response option. When a yes-response has been given, the participant is asked to rate on a 4-point scale how distressing the experience was to them. This version of the CAPE produces a frequency score and a distress score for each of the different symptom dimensions.

State Social Paranoia Scale (SSPS)

The SSPS is a 10-item instrument which measures persecutory thoughts (Freeman et al., 2007). The persecutory items (e.g. someone had bad intentions towards me) are presented among 10 neutral items and scored on a 5-point scale (0 = Do not agree – 5 = Totally agree).

The University of Wales Mood Adjective Checklist (UMACL)

The UMACL is used to measure the three major dimensions of affect (Matthews et al., 1990) : Hedonic tone (pleasure-displeasure), Energetic arousal (awake-tiredness), and Tense arousal (tension-relaxation). Each dimension consists of four negative and four positive adjectives of which the participant scores on a 4-point scale. The scores for each dimension are then added up and range from -12 to 12.

Beck's Anxiety Inventory

The Beck's Anxiety Inventory measures clinical symptoms of anxiety and consists of 20-items scaled on a 4-point scale (0 = Not at all – 5 = Severely) (Beck et al., 1988).

Visual analog scale (VAS)

Visual analog scales were used to measure the following feeling states: 'high', 'calm and relaxed', 'tired', 'anxious', and 'stoned'. The scale consists of a 100mm horizontal line on which the participant makes a vertical mark indicating how much of the feeling state he/she is experiencing, ranging from 'Not at all' to 'As much as could possibly be'.

Subjective effects

The pleasurable effects of the drugs were measured on a 5-point likert scale from "no" to "extreme" on the item "This experience is pleasurable". This was measured at baseline, post-capsule, and post-THC. After the completion of the 2nd experimental session, the participants were asked to name which one of the two THC-occasions they felt were the weakest or least intense.

Statistical analyses

All analyses were performed in SPSS 21 (IBM, N.Y.). Due to the small sample size of this study none of the data was normally distributed. Friedman's non-parametric repeated measures ANOVA was used to analyse differences between treatment weeks (1.Δ9-THCV 2.Placebo) across the testing points (1.Baseline, 2.Post-Capsule, 3.Post-Δ9-THC). Wilcoxon signed-rank test was used for post hoc analyses. Significance was accepted at $p < 0.05$, and all comparisons were two-tailed.

Results

At the end of the study six out of ten participants correctly guessed which week they had been given the Δ9-THCV capsules ($\chi^2=0.4$, $p=0.527$). Table 4.2 lists effects reported by study participants while taking either placebo or Δ9-THCV during the study weeks.

Table 4.2. Effects of Δ9-THCV and placebo

	Placebo	THCV
Tired	1	3
Trouble falling asleep	0	1
Nausea	0	1
Feeling active	2	0
Stiff neck/shoulder	2	0
Increased creativity	1	1

Cognition

Hopkins Verbal Learning Task

Immediate recall

There was no statistically significant change in number of words learned across sessions (χ^2=4.891, p=0.429). Post hoc analysis did not show any significant differences between post-capsule and post-Δ9-THC sessions under Δ9-THCV (Z=-1.349, p=0.177) or placebo condition (Z=-0.423, p=0.672).

Delayed recall

There was a statistically significant decrease in proportion of words recalled across sessions (χ^2=12.99, p=0.023). Post hoc analysis revealed a significant decrease in words recalled between post-capsule and post-Δ9-THC session under placebo condition (Z=-2.201, p=0.028), but not under Δ9-THCV condition (Z=-1.524, p=0.128) There was no significant difference between placebo and Δ9-THCV condition at the post-Δ9-THC session (Z=-1.153, p=0.249) (Figure 4.2 A).

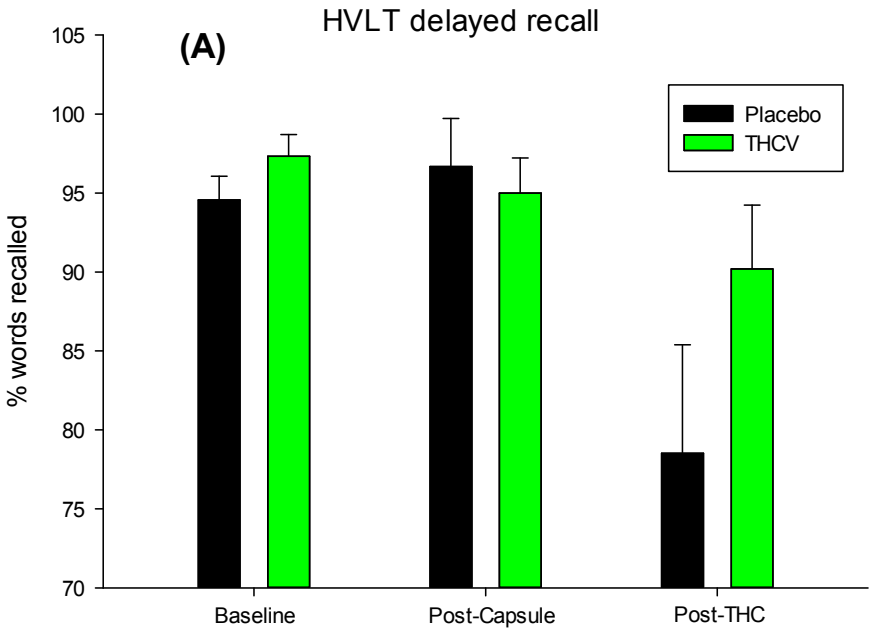
Repetitions

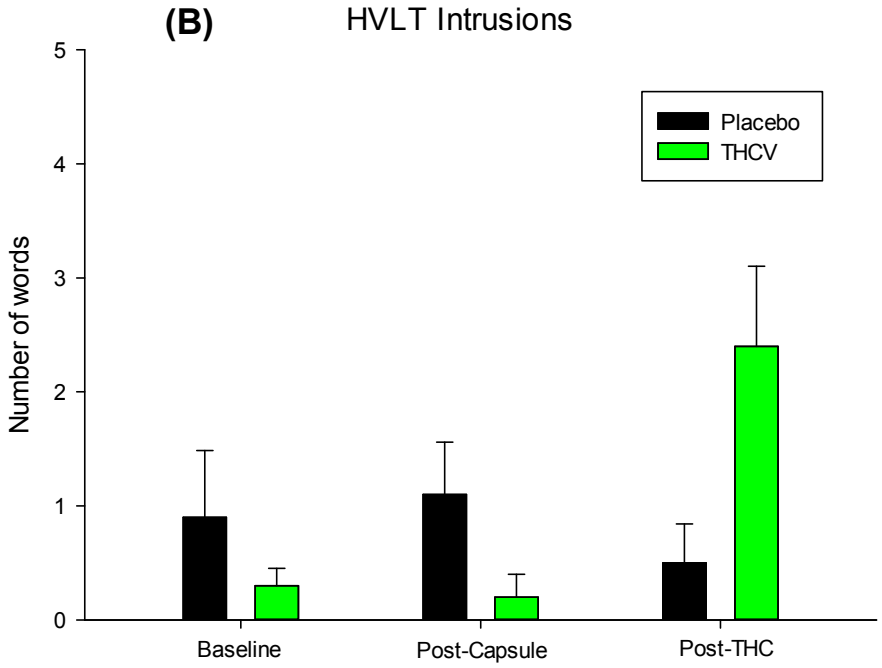
There was no statistically significant change in repetitions across sessions (χ^2=2.755, p=0.738).

Intrusions

There was a statistically significant increase in intrusions across session (χ^2=13.34, p=0.02). Post hoc analysis revealed no significant increase between post-capsule and post-Δ9-THC sessions under placebo condition (Z=-0.954, p=0.34), whereas there was a significant increase under Δ9-THCV condition (Z=-2.155, p=0.031). This suggests an interactive effect of Δ9-THCV and Δ9-THC to produce a significant increase in intrusions (Figure 4.2 B)

Figure 4.2 (A) Proportion of words recalled on the HVLT delayed recall across sessions (mean ± SEM). **(B)** Number of intrusions on the HVLT across sessions (mean ± SEM).





Digit-span forward

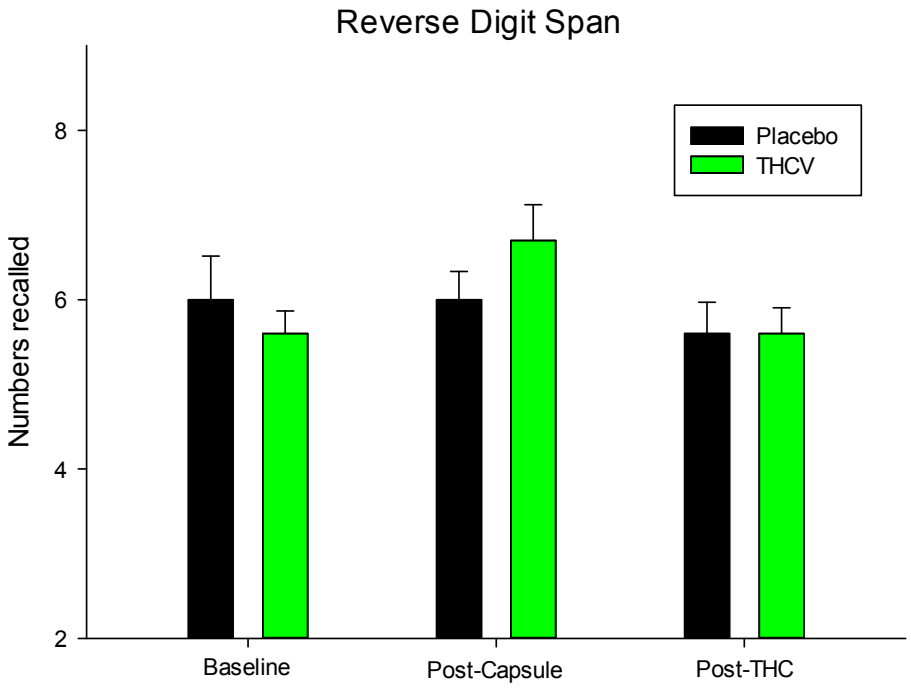
There was no statistically significant change in numbers recalled across sessions (χ^2=4.86, p=0.433). Post hoc analysis did not show any significant differences between

post-capsule and post-Δ9-THC sessions under Δ9-THCV (Z=-1.473, p=0.141) or placebo condition (Z=-1.508, p=0.132).

Digit-span reverse

There was no statistically significant change in numbers recalled across sessions (χ^2=8.642, p=0.124). Post hoc analysis revealed a significant improvement in reverse digit span performance between baseline and post-capsule and post-Δ9-THC sessions under Δ9-THCV condition (Z=-2.050, p=0.04) (Figure.4.3).

Figure.4.3 Reverse digit span performance across sessions (mean ± SEM).



Psychology

CAPE-state

Positive symptoms

There was no statistically significant change in positive symptom frequency across sessions (χ^2=8.305, p=0.14). Post hoc analysis revealed a statistically significant increase in positive symptoms between post-capsule and post-Δ9-THC sessions under placebo condition (Z=-2.00, p=0.046), but not under Δ9-THCV condition (Z=-1.342, p=0.18). This effect was not significant when comparing baseline and post-Δ9-THC

sessions under placebo condition (Z=-0.333, p=0.739) (Figure 4.4 A). There was no statistically significant change in positive symptom distress scores across sessions (χ^2=4.00, p=0.549).

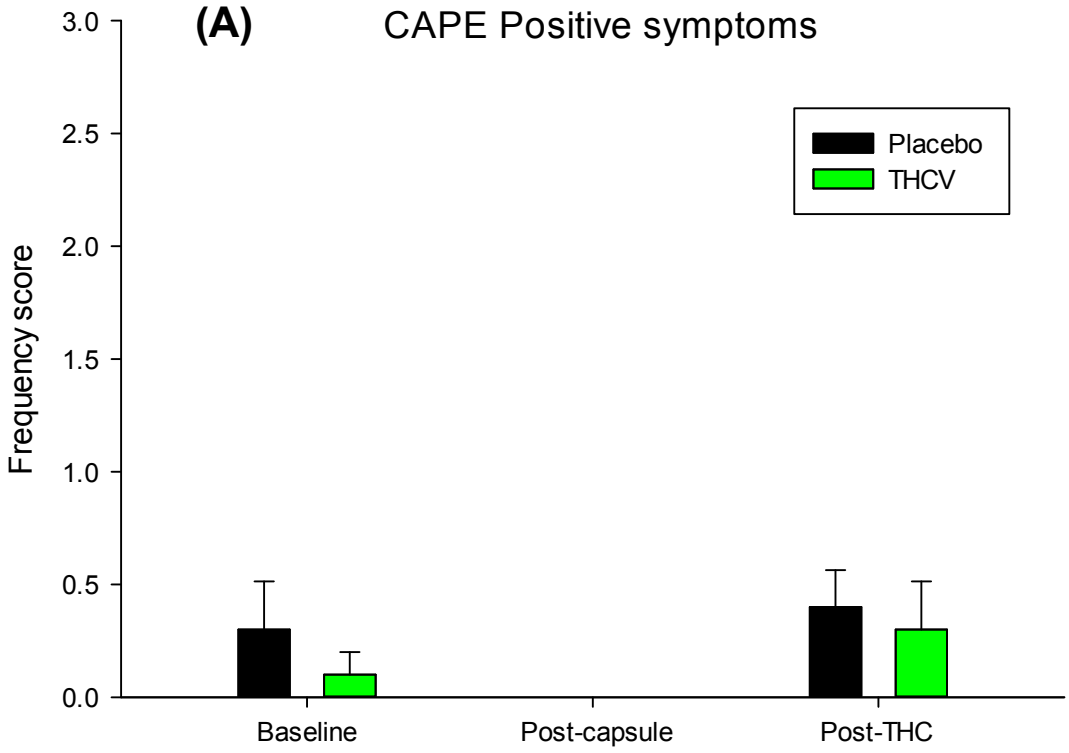
Depressive symptoms

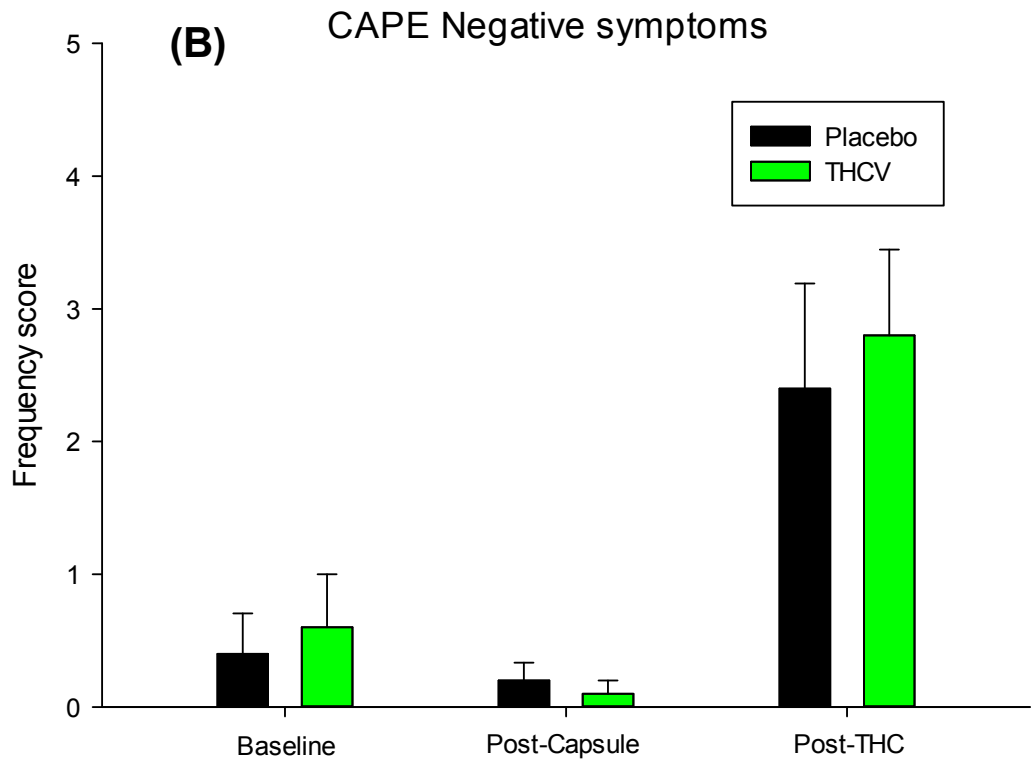
There was no statistically significant change in depressive symptom frequency across sessions (χ^2=5.612, p=0.346), or depressive symptom distress score (χ^2=5.00, p=0.416).

Negative symptoms

There was a statistically significant increase in negative symptom frequency across sessions (χ^2=22.716, p<0.001). Post hoc analysis revealed significant increases between post-capsule and post-Δ9-THC sessions under both placebo (Z=-2.328, p=0.02) and Δ9-THCV conditions (Z=-2.375, p=0.018) (Figure 4.4 B).

Figure 4.4 (A) Frequency of positive symptoms on the CAPE-state scale (mean ± SEM). **(B)** Frequency of negative symptoms on the CAPE-state scale (mean ± SEM).





SSPS

There was no statistically significant change in paranoia across sessions (χ^2=2.931, p=0.711).

UMACL

Hedonic tone

There was a statistically non-significant trend towards a change in hedonic tone scores across sessions (χ^2=18.619, p=0.068). There were no significant changes between post-capsule and post-Δ9-THC sessions under placebo (Z=0, p=1.00) and Δ9-THCV condition (Z=-0.352, p=0.725) (Figure 4.5 A).

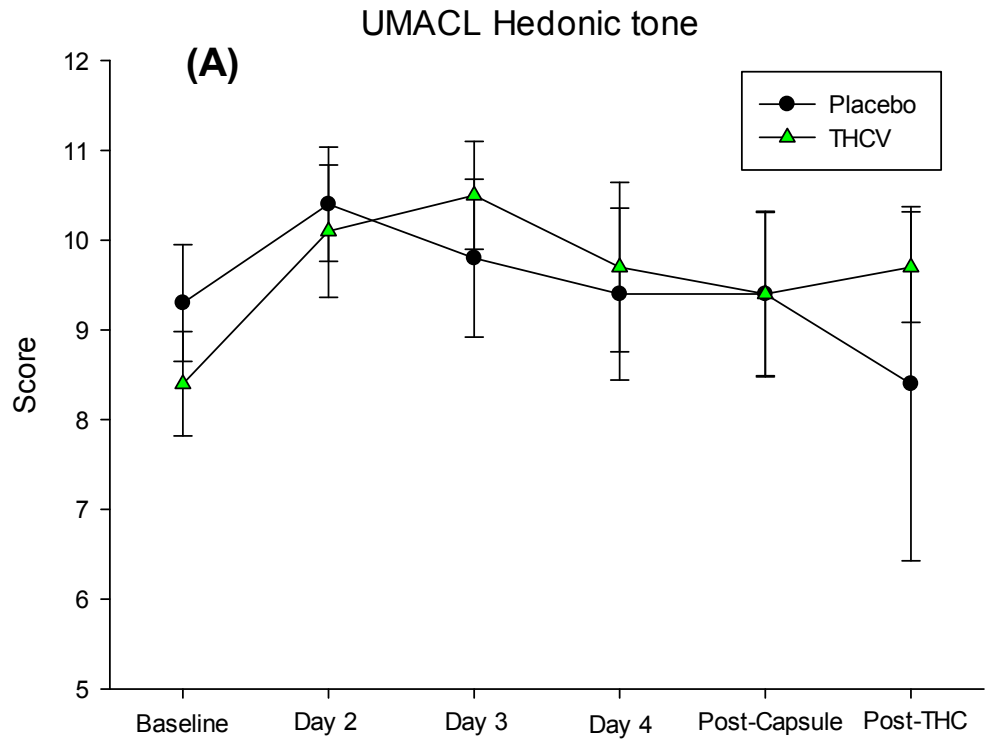
Energetic arousal

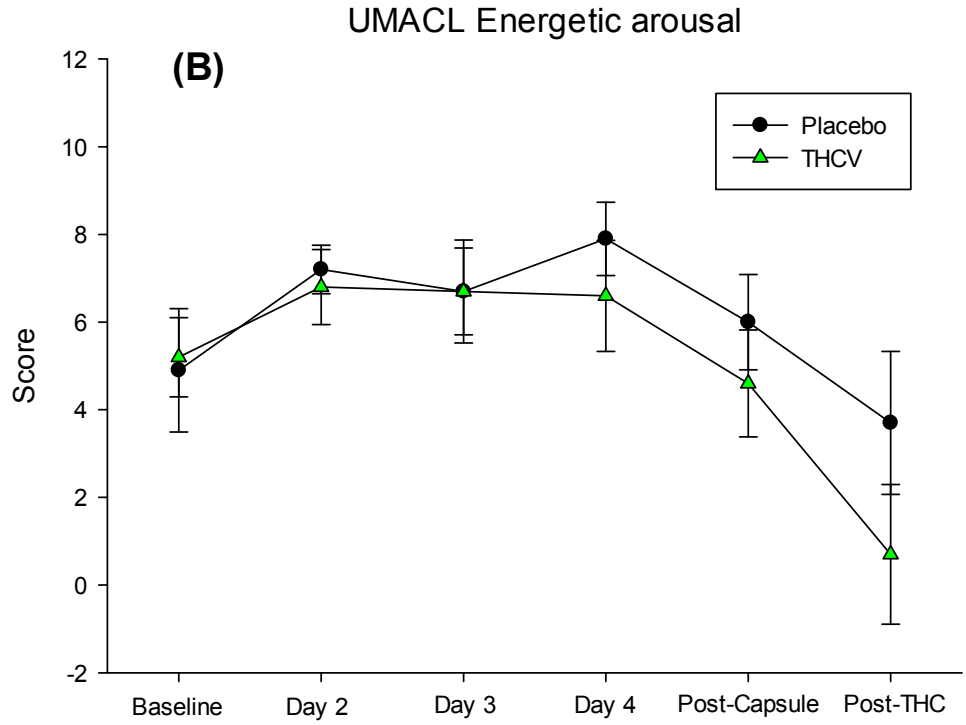
There was a statistically significant decrease in energetic arousal scores across sessions (χ^2=23.988, p=0.013). Post hoc analysis revealed a significant decrease between post-capsule and post-Δ9-THC sessions under Δ9-THCV condition (Z=-1.99, p=0.047), but not under placebo condition (Z=-1.011, p=0.312) (Figure 4.5 B).

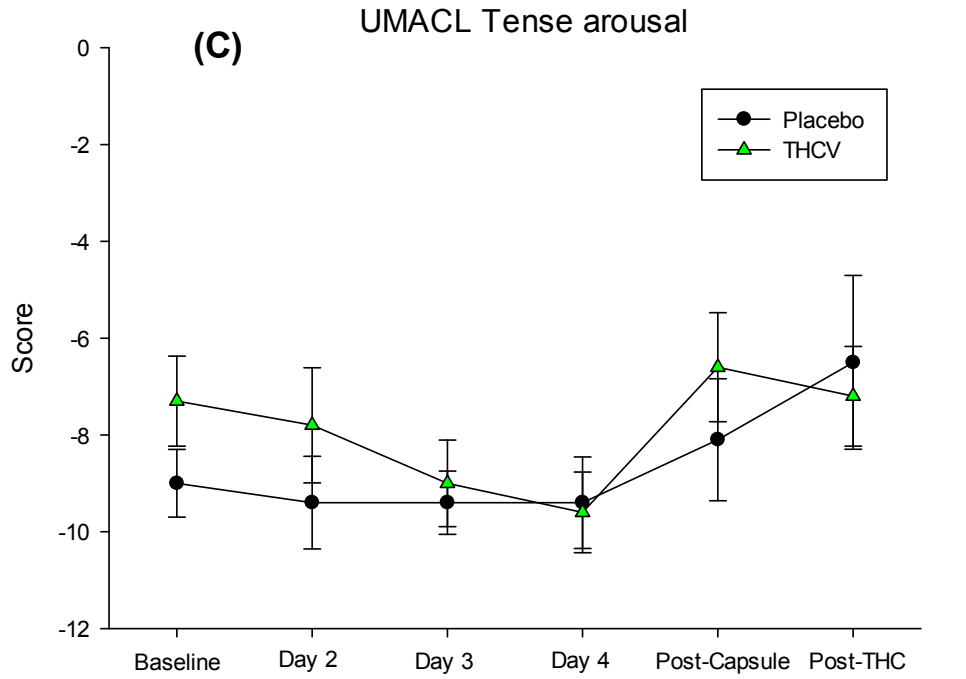
Tense arousal

There was no statistically significant change in tense arousal scores across sessions (χ^2=15.248, p=0.171). Post hoc analysis revealed a significant increase between Day 4 and post-capsule under Δ9-THCV condition (Z=-2.055, p=0.04), but not under placebo condition (Z=-1.166, p=0.244) (Figure 4.5 C).

Figure 4.5 (A) Scores on the hedonic tone subsection of the UMACL across sessions (mean ± SEM). **(B)** Scores on the energetic arousal subsection of the UMACL across sessions (mean ± SEM). **(C)** Scores on the tense arousal subsection of the UMACL across sessions (mean ± SEM).



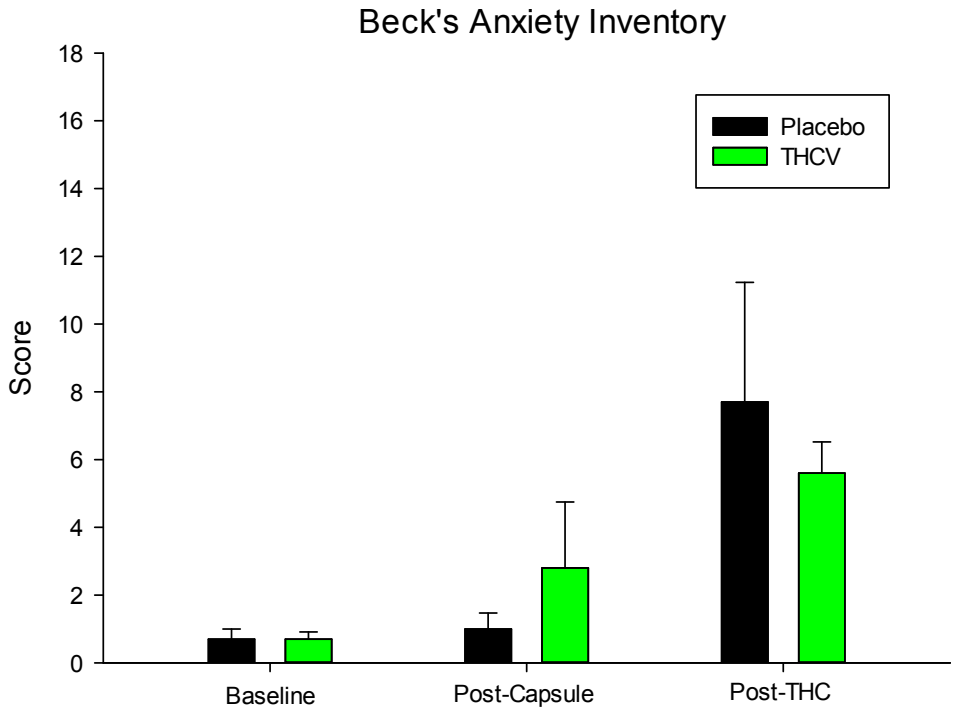

UMACL Energetic arousal
(B)
Placebo
THCV
Score
12
10
8
6
4
2
0
-2
Baseline
Day 2
Day 3
Day 4
Post-Capsule
Post-THC


UMACL Tense arousal
(C)
Placebo
THCV
Score
0
-2
-4
-6
-8
-10
-12
Baseline
Day 2
Day 3
Day 4
Post-Capsule
Post-THC

Beck's Anxiety Inventory

There was a statistically significant increase in anxiety scores across sessions (χ^2=31.097, p<0.001). Post hoc analysis revealed a significant increase of anxiety between post-capsule and post-Δ9-THC sessions under placebo condition (Z=-2.67, p=0.008), while this was at a trend level under Δ9-THCV condition (Z=-1.602, p=0.109). There were no significant differences of anxiety scores between placebo and Δ9-THCV conditions at the post-Δ9-THC session (Z=-0.543, p=0.587). There was no significant increase of anxiety between baseline and post-capsule under Δ9-THCV condition (Z=-1.289, p=0.197) (Figure. 4.6).

Figure 4.6 Scores on Beck's Anxiety inventory (mean ± SEM)



Visual analog scale

Anxious

There was no statistically significant increase in VAS anxiety scores across sessions (χ^2=4.792, p=0.442). Post hoc analysis revealed a non-significant increase in anxiety between baseline and post-capsule sessions under Δ9-THCV condition (Z=-1.521, p=0.128) (Figure 4.7 A).

Calm

There was no statistically significant increase in VAS calm scores across sessions (χ^2=3.481, p=0.626).

Tired

There was a non-significant trend towards increased VAS tired scores across sessions (χ^2=9.568, p=0.088). Post hoc analysis revealed a non-significant trend towards increased tired scores between post-capsule and post-Δ9-THC session under Δ9-THCV condition (Z=-1.682, p=0.093), but not under placebo condition (Z=-1.362, p=0.173).

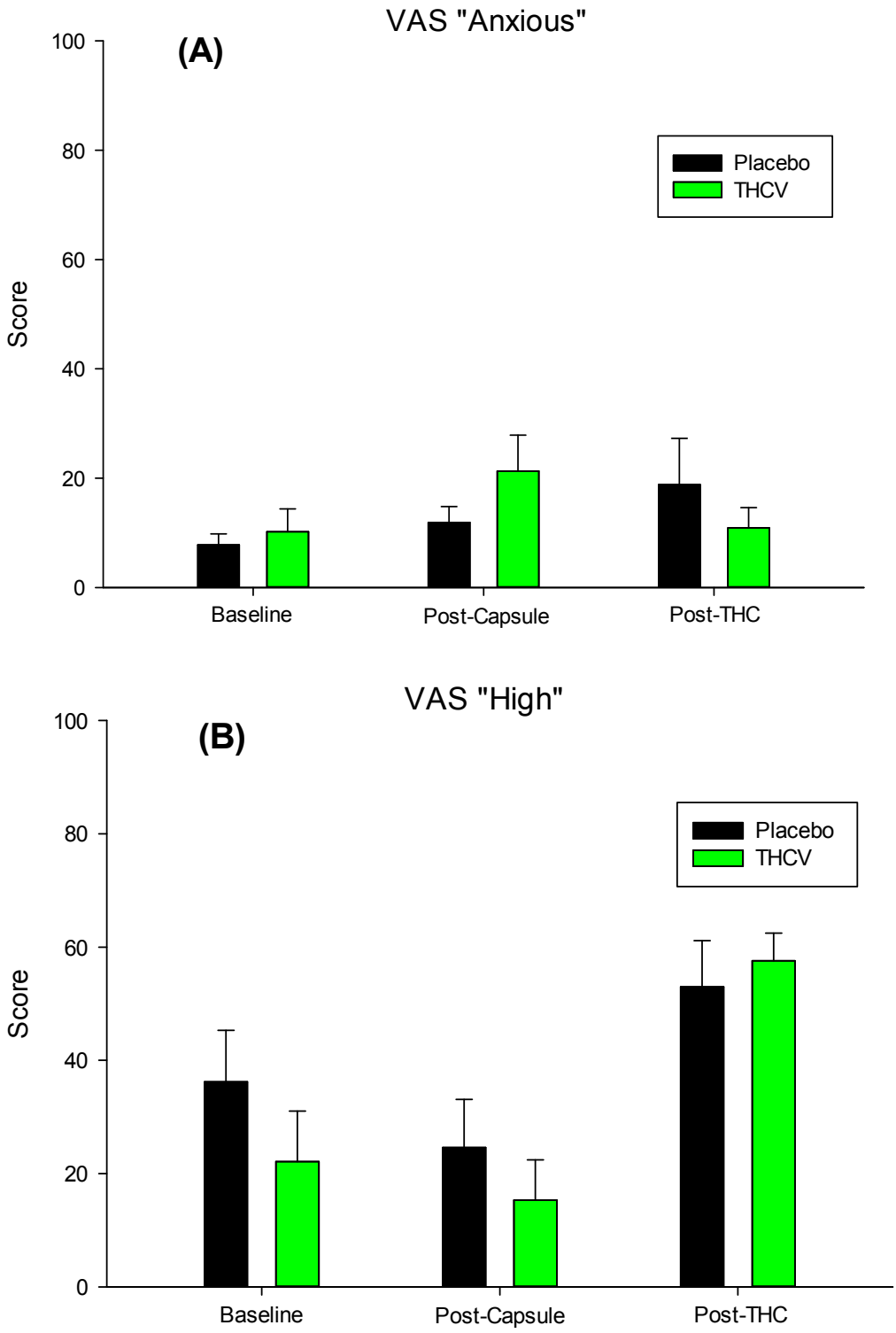
High

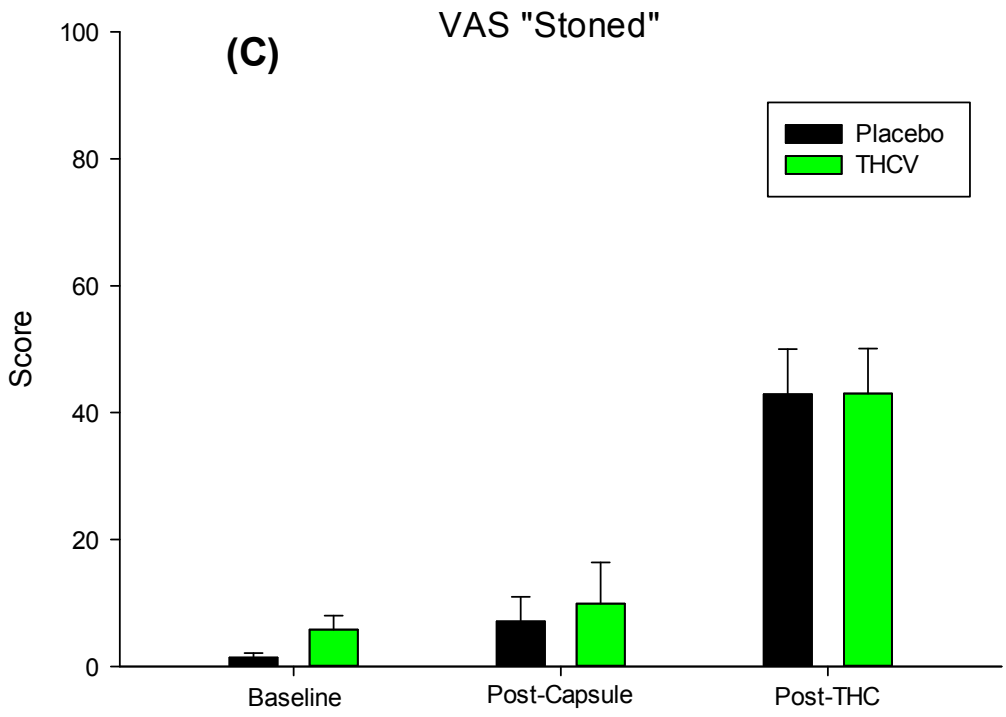
There was a statistically significant increase in VAS high scores across sessions (χ^2=20.26, p=0.001). Post hoc analysis did not show any significant difference between placebo and Δ9-THCV conditions at the post-Δ9-THC session (Z=-0.153, p=0.878) (Figure 4.7 B).

Stoned

There was a statistically significant increase in VAS stoned scores across sessions (χ^2=33.626, p<0.001). Post hoc analysis did not show any significant difference between placebo and Δ9-THCV conditions at the post-Δ9-THC session (Z=-0.051, p=0.959) (figure 4.7 C).

Figure 4.7 (A) Scores on visual analog scale for "anxious" (mean ± SEM)**. (B)** Scores on visual analog scale for "high" (mean ± SEM). **(C)** Scores on visual analog scale for "stoned" (mean ± SEM).

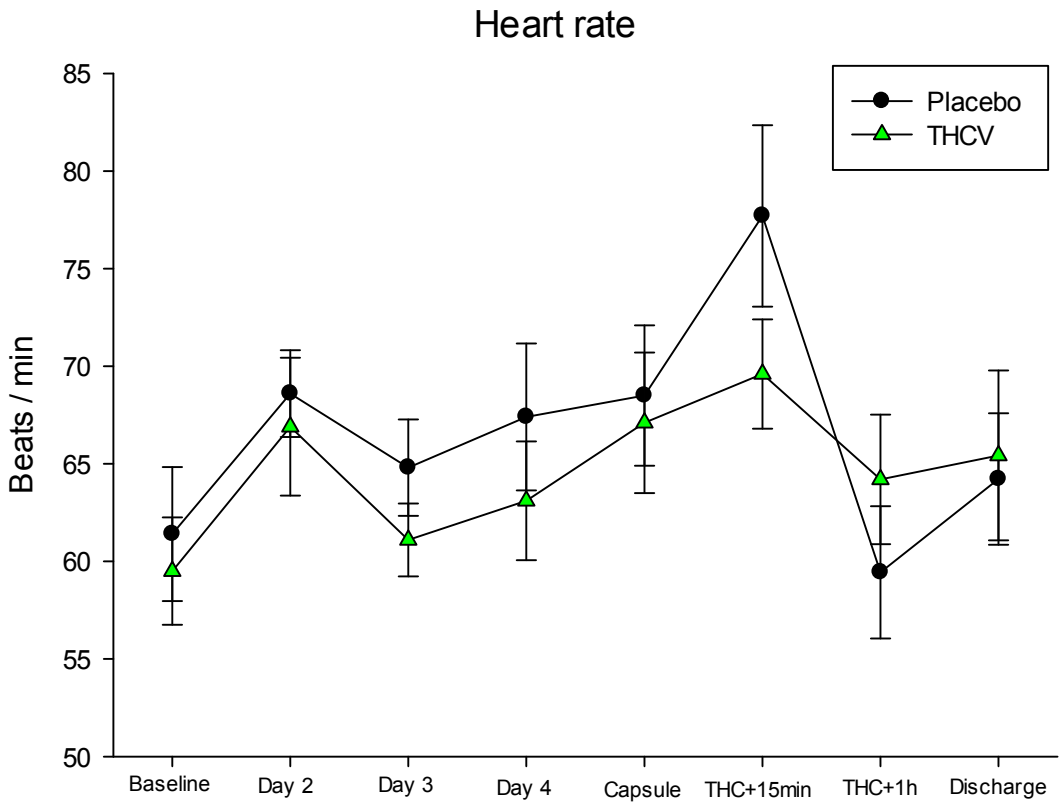



(C)
VAS "Stoned"
Placebo
THCV
Score
100
80
60
40
20
0
Baseline
Post-Capsule
Post-THC

Cardiovascular

There were no significant changes across sessions in systolic (χ^2=20.938, p=0.139) or diastolic (χ^2=14.444, p=0.492) blood pressure. There was a significant change in heart rate across sessions (χ^2=27.019, p=0.029). Post-hoc analysis revealed a significant increase in heart rate at the Δ9-THC+15min testing point under placebo condition compared to Δ9-THCV condition (Z=--2.193, p=0.028) (Figure 4.8).

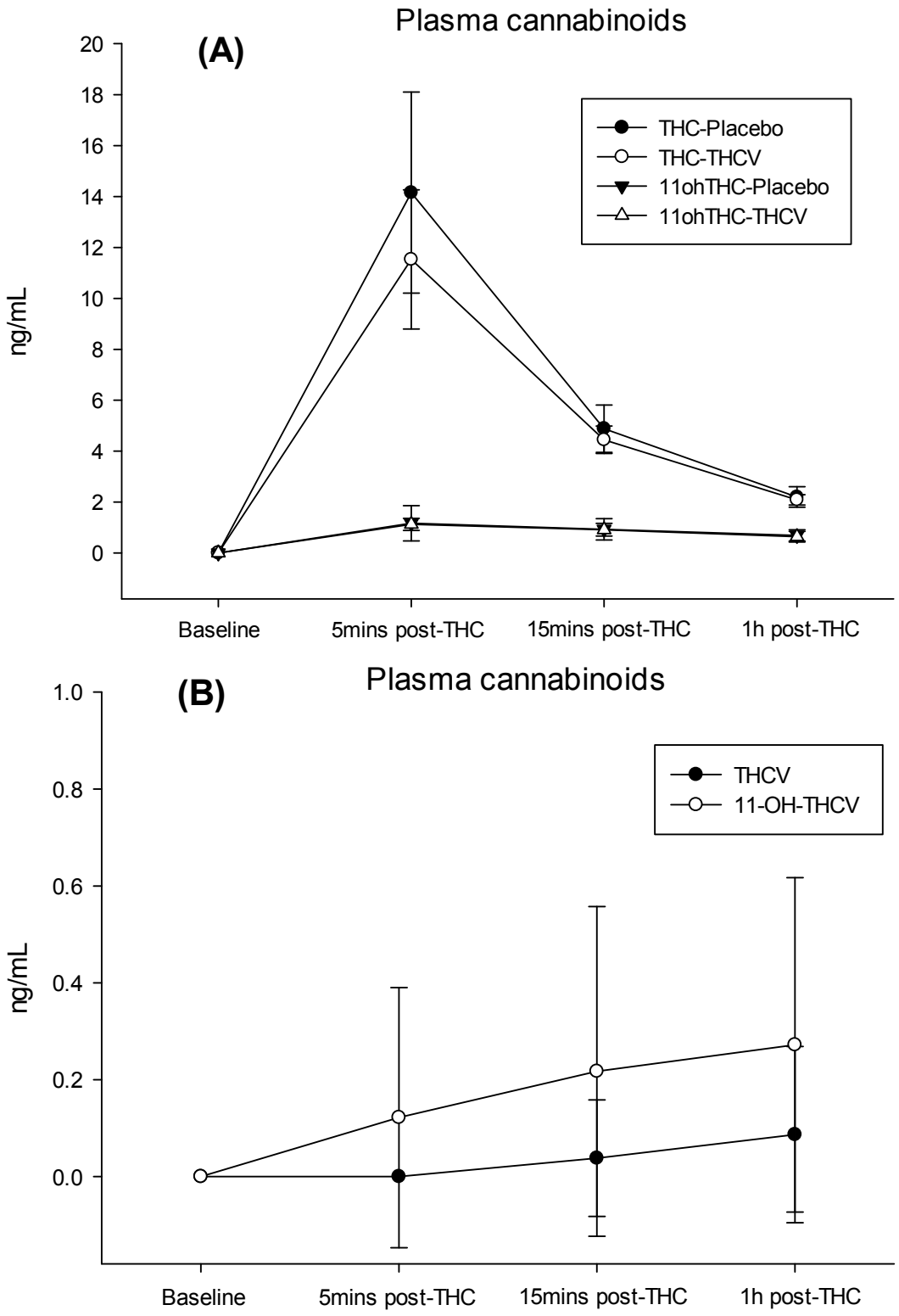
Figure 4.8 Heart rate in beats/min (mean ± SEM).



Pharmacokinetics

The plasma concentrations of Δ9-THC, 11-OH-Δ9-THC under placebo and Δ9-THCV condition, as well as Δ9-THCV and 11-OH-Δ9-THCV are presented in Figure 4.9 A. There were no significant differences between placebo and Δ9-THCV conditions in Δ9-THC concentrations at 5 min post-Δ9-THC ($Z=-1.355$, $p=0.176$) and 1 hour post-Δ9-THC ($Z=-1.051$, $p=0.293$). There was a trend towards higher Δ9-THC plasma concentrations under placebo condition at the 15 min post-Δ9-THC session ($Z=-1.718$, $p=0.086$). Plasma Δ9-THCV was only above limit of quantification (ALQ, >0.25 ng/mL) in 3 out of 37 samples, while its main metabolite 11-OH-Δ9-THCV was detectable in 11 out of 37 samples (Figure 4.9 B).

Figure 4.9 (A) Plasma cannabinoid concentrations of Δ9-THC and 11-OH-Δ9-THC under placebo and Δ9-THCV conditions (mean ± SEM). **(B)** Plasma cannabinoid concentrations of Δ9-THCV and 11-OH-Δ9-THCV (mean ± SEM).



Subjective effects

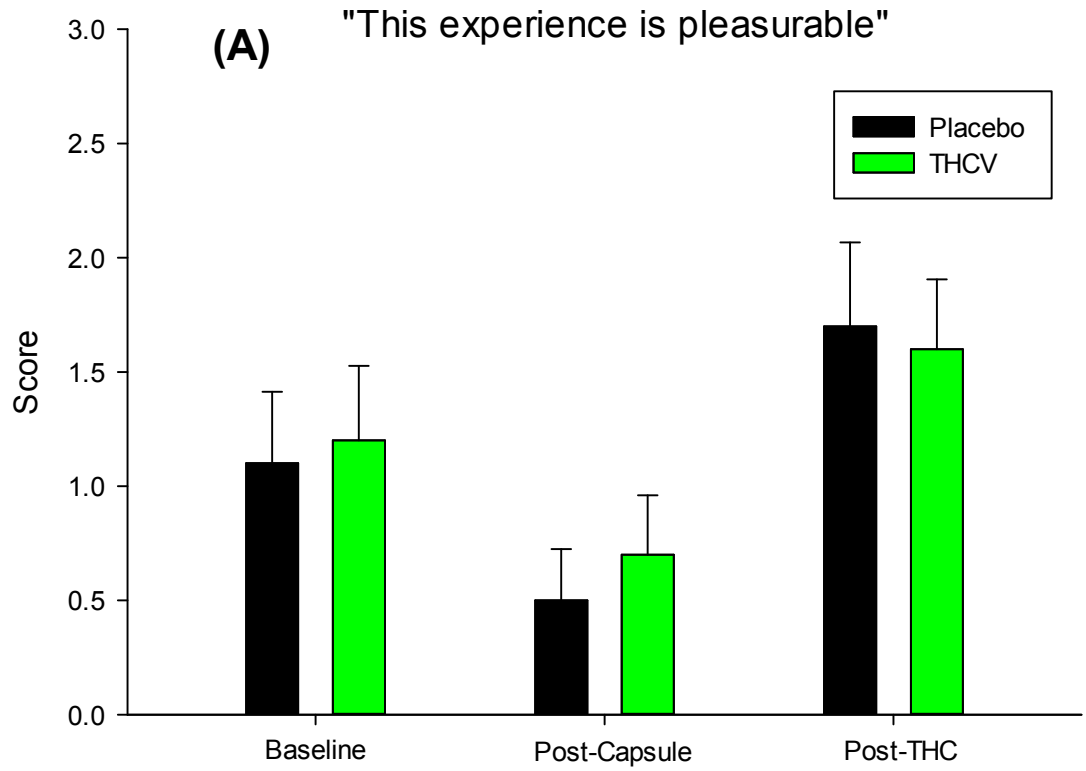
Pleasure

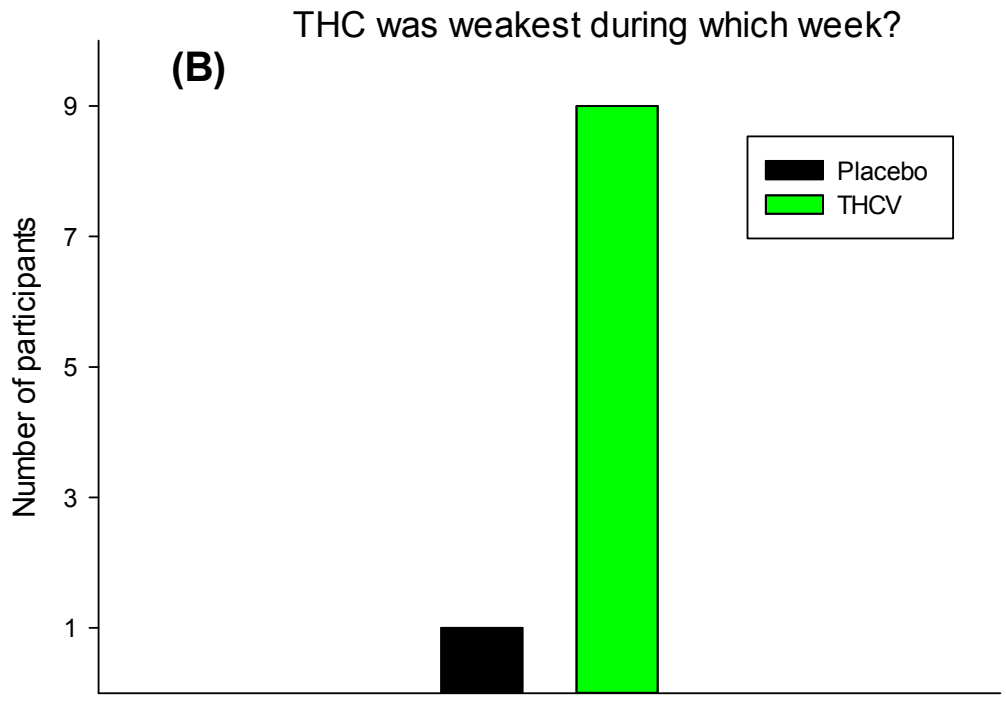
There was a statistically significant increase in pleasure scores across sessions (χ^2=16.033, p=0.007). Post hoc analysis did not show any significant difference between placebo and Δ9-THCV conditions at the post-Δ9-THC session (Z=-0.172, p=0.863) (figure 4.10 A).

Δ9-THC strength

Nine out of ten participants reported Δ9-THC as being either weaker or less intense under Δ9-THCV condition (χ^2=6.4, p=0.011) (Figure 4.10 B).

Figure.9 (A) Subjective rating of pleasure across sessions (mean ± SEM). **(B)** Subjective report of the weaker Δ9-THC experience.





Discussion

To our knowledge this is the first study exploring the interactive effects of Δ9-THCV and Δ9-THC in healthy volunteers. Δ9-THCV was well tolerated among the participants and no serious adverse effects were observed. In fact, participants could not significantly distinguish the Δ9-THCV capsules from the placebo capsules. However, during the Δ9-THCV week, three participants reported feeling more tired than usual, while this only occurred for one participant during placebo treatment.

Cognition

Interestingly the low dose of 1mg IV Δ9-THC did not produce any significant memory impairment on either the HVLT immediate recall or Digit Span task. These results highlight the previously shown dose-dependent effects of Δ9-THC (Naef et al., 2004), e.g. where higher doses of Δ9-THC have been found to cause anxiety (D'Souza et al., 2004) while lower doses reduce it (Phan et al., 2008). It has been proposed that this may either be due to Δ9-THC acting as a CB1 antagonist at higher doses (Pertwee, 2008) or disruption of endocannabinoid mediated neuronal firing (Morrison et al., 2011). There was however a significant drop in performance on HVLT delayed recall, an effect that was only present during placebo treatment following Δ9-THC. This drop in performance has been well documented in other studies with Δ9-THC, and represents impairment in consolidation and retrieval of episodic information from long term memory (D'Souza et al., 2005; Morgan, Schafer, et al., 2010). However, previous studies have shown Δ9-THC-induced short-term memory impairments to immediate recall as well as impairments to consolidation and retrieval, while in this study only consolidation and retrieval were negatively affected. This may suggest that consolidation and retrieval of episodic memory are more vulnerable to the effects of Δ9-THC than short-term memory. In previous Δ9-THC studies, CBD has been shown to protect against impairments to episodic delayed recall (Englund et al., 2013; Morgan, Schafer, et al., 2010). Although Δ9-THC did produce impairments to delayed recall, this effect was only present under placebo treatment and absent in the presence of Δ9-THCV. This suggests a similar protective and antagonistic effect of Δ9-THCV on Δ9-THC induced memory impairment.

A surprising result was that of a small but significant improvement in performance on the Reverse Digit Span task, where Δ9-THCV improved performance compared to baseline; while this effect was absent during placebo condition. It is especially surprising since no improvements were observed in either the Forward Digit Span task or during immediate recall of the HVLT. Since the Reverse Digit Span task requires participants to recall the reverse order of a list of digits, it puts a higher demand on working memory capacity. Not only do the participants need to hold the information in short-term memory, but they also need to manipulate the digits to give the correct order. Previous animal studies have shown improvements to working memory following either administration of CB1 antagonists (Terranova et al., 1996; Lichtman, 2000), or genetic deletion of the CB1 receptor (Reibaud et al., 1999). However, there are also contradictory findings in the literature where administration of CB1 antagonists such as Rimonabant have either had no effect on cognition (Hampson and Deadwyler, 2000) or an impairing effect (Mallet and Beninger, 1998). Varvel and colleagues have argued that this disparity in the literature may be explained by the differences in tasks employed, suggesting that CB1 antagonists may facilitate retention for longer while having no effect on short term retention (Varvel et al., 2009). This is contrary to our findings as I found only a significant improvement on the Reverse Digit Span task which requires a response from the participant immediately. Furthermore, I observed no improvement in long-term memory as measured by the delayed recall of the HVLT.

Interestingly, I observed a significant interaction between Δ9-THC and Δ9-THCV, which produced an increase in intrusions. This effect was not observed under any of the other conditions. Intrusions are errors in memory thought to reflect a lack of inhibition of semantically related information (Schacter et al., 1998). A study in amnesic patients found that intrusions were correlated with verbal fluency (Schnider et al., 1996) which is commonly a measure for creativity. Previous studies with high dose IV Δ9-THC have reported no significant change in verbal fluency in healthy volunteers (D'Souza et al., 2004; Morrison et al., 2009), while reporting an increase in intrusions. However, these studies all reported impairments to immediate and delayed recall performance, while in the current study participants were not impaired following IV Δ9-THC. In a naturalistic study, where participants smoked their own cannabis at home, there was a

significant increase in verbal fluency after smoking amongst participants low in creativity (Schafer et al., 2012). The low dose used in the current study is more likely to reflect that of the naturalistic study. Hence, it could be argued that the increase in intrusions following the combination of Δ9-THC and Δ9-THCV reflects a similar increase in creativity and lacking inhibition, considering the fact that performance was not impaired in any of the tested domains. Of course, this remains speculative since verbal fluency was not tested in this study.

Psychological effects

Similarly to the effects on cognition, the dose of 1mg IV Δ9-THC did not produce any significant positive psychotic symptoms or paranoia as has been previously reported with higher doses of Δ9-THC (D'Souza et al., 2004; D'Souza, Ranganathan, et al., 2008; Morrison et al., 2009, 2011; Englund et al., 2013). In a recent review, I argued that previous studies with IV Δ9-THC have administered doses which more reflected an over-intoxication of cannabis rather than reflecting recreational cannabis use (Englund et al., 2012). The results of the current study are in agreement with this hypothesis. There was a significant increase of negative symptoms on the CAPE scale, regardless of Δ9-THCV or placebo condition, following Δ9-THC; something which has been demonstrated with higher doses of Δ9-THC (Morrison and Stone, 2011). The most endorsed items were "Do you feel that you are not very animated" and "Do you feel that you are lacking in energy". These results are in line with the post Δ9-THC reduction in UMACL Energetic arousal scores in both conditions, and is something that has been observed in other Δ9-THC studies (Morrison et al., 2009).

An interesting trend which emerged was that of apparent increased anxiety at the post capsule testing point, only under Δ9-THCV condition. This trend was observed in the Beck's anxiety inventory, VAS anxiety, and UMACL tense arousal. Although there was no significant increase across sessions on VAS anxiety, there was a trend towards an increase from baseline to post capsule testing points under Δ9-THCV condition. Similarly on the UMACL Tense arousal, there was no increase of anxiety across session with the exception of a significant increase between day 4 and post capsule testing points under Δ9-THCV condition. On the Beck's scale, there was a significant increase of anxiety across session, most likely due to more items relating to the physiological

symptoms of anxiety. Similarly, there was a slight increase in anxiety from Δ9-THCV between baseline and post-capsule testing points, although this relationship was very weak. Seen together, these results seem to indicate a potential, yet weak, anxiogenic effect of Δ9-THCV alone. However, it is important to remember that these observed small increases in anxiety occurred at the post-capsule testing point which is on the same day as IV Δ9-THC administration, where the anticipation of this might provoke anxiety. This is highlighted in the UMACL Tense arousal, where there was an increase at post-capsule testing point compared to Day 3 and 4. Although, as this effect is absent under placebo condition, Δ9-THCV may make participants more sensitive to anxiogenic events or stimuli. It is important to note that previous research into the effects of CB1 antagonists in healthy volunteers have observed no change to subjective mood while showing a significant bias towards negatively loaded words (Horder et al., 2009, 2012). Future research into the potential anxiogenic potential of Δ9-THCV would benefit from employing such tasks to better observe such subtle changes in mood.

Subjective effects

Following completion of both Δ9-THC sessions, participants were asked which Δ9-THC session they felt was the weakest or least intense. Nine out of ten reported the Δ9-THC session under Δ9-THCV condition to be the weaker/less intense experience, suggesting Δ9-THCV has a significant impact on the subjective intensity of IV Δ9-THC. Furthermore, I asked participants to rate on a 4 point scale how pleasurable they perceived the experience. Δ9-THC significantly increased how pleasurable the experience was with no difference between placebo and Δ9-THCV condition, suggesting that Δ9-THCV does not impact on the pleasurable effects of Δ9-THC.

Cardiovascular and Pharmacokinetics

The most common cardiovascular effects of Δ9-THC in humans are tachycardia, vasodilatation, increased cardiac output and variable changes to blood pressure (Dewey, 1986). In the present study I observed a high variation in both systolic and diastolic blood pressure following IV Δ9-THC. Some participants became momentarily hypertensive, while others became hypotensive. This observation sheds some doubt on some small but significant differences found in both systolic and diastolic blood

pressure. Tachycardia is a more robust effect observed in this study following Δ9-THC administration. Interestingly, this effect was blocked by Δ9-THCV suggesting a pharmacological inhibition of the peripheral heart rate effects of Δ9-THC.

The main pharmacokinetic finding in this study was that Δ9-THCV did not significantly change plasma levels of Δ9-THC, suggesting that Δ9-THCV does not affect the bioavailability of Δ9-THC while still producing measureable changes to its effects. Strangely, only 3 out of 37 plasma samples showed levels above quantification. This might be due to particularly rapid redistribution of Δ9-THCV, or potentially due to the timing of capsule administration. Future studies would benefit from taking these factors into consideration and perform more frequent plasma sampling following Δ9-THCV administration to elucidate its pharmacokinetics. I did however observe significantly more samples showing quantifiable levels of the Δ9-THCV metabolite 11-OH-Δ9-THCV (11 out of 37 samples), indicating the recent presence of Δ9-THCV.

Pharmacological mechanisms

The pharmacological actions of Δ9-THC have been well established and are mainly comprised of partial-agonism at the CB1 receptor. As mentioned previously, other pharmacological actions of Δ9-THC are also likely (Pertwee, 2008), although the extent to which they influence behavioural effects remain unclear.

I have previously noted that Δ9-THCV acts as a neutral antagonist in the lower dose-range (Wargent et al., 2013) while acting as a weak agonist at higher doses (Hollister, 1974). The current study supports the notion of Δ9-THCV as a competitive antagonist, as impairments to delayed verbal recall and heart rate increase were successfully inhibited by Δ9-THCV. However, the interaction of both Δ9-THC and Δ9-THCV to significantly increase memory intrusions indicate a possible potentiating effect of Δ9-THCV on specific domains of cognition.

Strengths and Limitations

As the present study was a pilot study comprised of only 10 volunteers, this significantly impacts on the conclusions that may be drawn from this study. Due to the high variation in response to cannabinoids (Atakan, 2012; Englund et al., 2013; Morrison and Stone, 2011; D'Souza, Ranganathan, et al., 2008), it is likely that this

study was underpowered to capture the variation in responses to Δ9-THCV, with and without the presence for Δ9-THC. Furthermore, the decision of timing and dosage of Δ9-THCV were based on limited information as pharmacokinetic data of Δ9-THCV in humans is lacking. This may explain why so few plasma samples showed levels above quantification, although rapid redistribution of Δ9-THCV still remains a possible explanation. Lastly, the aim of this study was to explore the potential protective effects of Δ9-THCV on Δ9-THC-induced psychotic and paranoid symptoms. This was prevented as the lower dose of 1mg IV Δ9-THC did not produce significant symptoms. Future studies should explore the effects of Δ9-THCV using a higher dose of Δ9-THC.

Controlled laboratory experiments using pure and isolated cannabinoids benefit from reducing inter-individual variation to explore specific psychopharmacological interactions. Intravenous administration of Δ9-THC further reduces differences in bioavailability between subjects as oral and inhaled administration suffers from poor bioavailability and high inter-individual variation (Grotenhermen, 2003). Furthermore, the within subject design of the study particularly benefits cannabinoid studies as it reduces variation between subjects, which otherwise would require a larger sample size. Although this study only recruited participants who had used cannabis 25 times or less to minimise variation between subjects, future studies would benefit from also recruiting frequent users as they respond differently to cannabinoids (D'Souza, Ranganathan, et al., 2008).

Conclusion

In this small pilot study with healthy infrequent cannabis users, results indicate that the dose of 10mg oral Δ9-THCV is well tolerated with no serious adverse reactions and is subjectively not distinguishable from placebo. Furthermore, the lower dose of 1mg IV Δ9-THC did not produce any significant short-term memory impairment, as well as psychotic or paranoid reactions. Δ9-THCV significantly inhibited Δ9-THC-induced impairment to delayed recall as well as Δ9-THC-induced increase of heart rate. Δ9-THCV on its own showed signs towards improved performance on the harder working-memory task while also producing a slight increase in anxiety. However, these effects were small and need to be further studied in a larger sample.

Summary and Discussion

Summary of findings

In the first experiment, I demonstrated that CBD significantly inhibited Δ9-THC-induced paranoid thoughts and impairments to delayed verbal recall. The number of clinically significant psychotic reactions induced by Δ9-THC, as measured by the PANSS, was significantly reduced by CBD. Furthermore, performance on all working memory tasks (digit span forward, reverse, and immediate verbal recall) was impaired by Δ9-THC, an effect CBD did not protect against. However, processing speed and executive function were not affected by Δ9-THC.

Secondly, I showed that CBD did not have an effect on Δ9-THC-induced reductions to theta amplitude and coherence. However, delta amplitude was significantly increased following Δ9-THC administration, an effect that was inhibited by co-administration of CBD. Furthermore, alpha amplitude increased for each testing point, although the combination of Δ9-THC and CBD inhibited further increases of alpha. Lastly, neither theta nor alpha coherence was correlated with change in psychotic symptoms on either the PANSS or SSPS.

Finally, in a small pilot study, I found that a lower dose of IV Δ9-THC (1mg) did not significantly increase paranoia, positive psychotic symptoms or impair working memory in healthy volunteers. Delayed verbal recall was impaired by Δ9-THC, although this effect was blocked by pre-treatment with Δ9-THCV. Also, Δ9-THC-induced heart rate increase was inhibited by Δ9-THCV. On its own, Δ9-THCV improved performance on one of the working memory tasks and increased anxiety scores, although these effects were small.

How results relate to each other

From the above mentioned results it seems there potentially lays a threshold level of Δ9-THC between the two IV doses given (1mg and 1.5mg) in the two studies - a threshold which when crossed can result in psychotic symptoms and impairments to short-term memory. In the first study 1.5 mg IV Δ9-THC produced positive psychotic symptoms and impairments to immediate verbal recall and digit span. However, in the

second study, with the reduced dose of 1mg IV Δ9-THC, these effects were absent. Potentially, this might mean that certain doses of Δ9-THC are cognitively impairing and psychotogenic, while others are not. However, the second study was merely a small pilot study and a larger study would be needed for these results to be verified.

In both studies, co-administration of either CBD or Δ9-THCV significantly protected against the impairing effects of Δ9-THC on delayed verbal recall. This finding may suggest that both CBD and Δ9-THCV share similar pharmacological properties which act to inhibit the memory impairing effects of Δ9-THC. This would most probably be the ability of these cannabinoids to antagonise other agonists of the CB1 receptor, as Δ9-THCV is not known to increase endocannabinoid activity. Although it was clear that CBD showed significant protective properties against the psychotogenic and cognitively impairing effects of Δ9-THC, these were less evident in the EEG data. Δ9-THC significantly reduced theta coherence and amplitude regardless of pre-treatment group. However, CBD did significantly inhibit Δ9-THC-induced increases to delta amplitude, which could potentially relate to its protective effects on the behavioural data. Furthermore, there were inconsistencies in the findings concerning the working memory tasks between the two analyses. The behavioural study showed poorer performance after Δ9-THC on immediate verbal recall and digit span, while during EEG recording performance on the n-back task was unchanged. This might be explained by the differences between the tasks. The n-back task requires the participant to remember and refresh three items (3-back), while digit span and immediate verbal recall has up to ten. It may be that previous practice on these tasks prevents a drop in performance for the n-back tasks, while digit span and verbal recall remain vulnerable.

Alpha amplitude was increased after each subsequent testing point, most likely due to participant fatigue. Δ9-THC further enhanced this effect, while the combination of both Δ9-THC and CBD inhibited further alpha increase. Since alpha amplitude is thought to be a sign of neural inhibition, it is therefore possible that Δ9-THC does not inhibit neural activity when also taken with CBD. However, this effect was not observed in the theta band which is commonly associated with better performance on memory tasks. Also, only delayed verbal recall was protected by CBD while working memory functions were not.

An interesting observation in the EEG data was the apparent lack of effect of CBD on measures of amplitude and coherence. These results mimic the ones from the behavioural data where CBD on its own had no significant effect on psychological and cognitive measures. This is in line with the knowledge of CBD as an endocannabinoid enhancer, and the endocannabinoid function as an "on demand" system which lays dormant if remained unchallenged.

Both CBD and Δ9-THCV appear to have no psychoactive effects when taken on their own and in the doses mentioned above (CBD: 600mg, Δ9-THCV: 10mg/daily). Δ9-THCV however did produce a slight increase in anxiety, although this was only seen on the 5^{th} day of administration, the day of the experiment, which might indicate that Δ9-THCV increases sensitivity to stressors. However, the slight increase in anxiety by Δ9-THCV was reduced by IV Δ9-THC administration.

How my results relate to other research findings

In this thesis I have replicated the findings of previous IV Δ9-THC studies which found that Δ9-THC significantly induces positive psychotic symptoms in healthy volunteers (Morrison et al., 2009; D'Souza et al., 2004, 2005; D'Souza, Ranganathan, et al., 2008; D'Souza, Braley, et al., 2008; Barkus et al., 2011; Morrison et al., 2011; Stone et al., 2012). The rate of participants experiencing psychotic reactions from Δ9-THC in these studies ranged from 40-50%. In the first study of this thesis, the rate was 42%, which is in line with previous work and strengthens the notion of the psychotogenic effects of Δ9-THC. It also supports individual resilience towards Δ9-THC in roughly 50% of the healthy population. Interestingly, in the second study of this thesis, the lower dose of 1mg IV Δ9-THC did not produce significant positive psychotic symptoms or paranoia. Previous studies have demonstrated that lower doses of inhaled Δ9-THC did not produce anxiety or psychosis (Phan et al., 2008; Naef et al., 2004; Brenneisen et al., 2010), but this is the first study to measure psychotic symptoms at the IV dose of 1mg.

I have also replicated the finding that CBD counteracts the psychotogenic effects of Δ9-THC (Di Forti et al., 2009; Morgan and Curran, 2008; Schubart et al., 2011; Leweke et al., 2000), and highlighted the specific anti-paranoia effects of CBD. In a clinical trial of CBD as an anti-psychotic medication for schizophrenia, Leweke and colleagues demonstrated that CBD significantly increased levels of anandamide (AEA) (Leweke et

al., 2012). Furthermore, they found that the levels of serum AEA were negatively correlated with positive psychotic symptoms. This is in line with their previous research where they showed that schizophrenic patients with higher CSF (cerebro spinal fluid) AEA had fewer positive psychotic symptoms (Giuffrida et al., 2004) and prodromal patients with higher levels transition later into psychotic illness (Koethe et al., 2009). In a recent study of CSF AEA levels of healthy cannabis users, the authors reported significantly lower levels of AEA in heavy users compared to light users. They also reported that levels of AEA were negatively correlated with psychotic symptoms while not under the influence of cannabis (Morgan et al., 2013), again highlighting the anti-psychotic effects of increased endocannabinoid signalling. Together, these studies suggest it is the increased levels of AEA that are responsible for the anti-psychotic effects of CBD in this thesis. However, CBD is also known to strongly antagonise other CB1 agonists via an indirect mechanism (Thomas et al., 2007; Pertwee et al., 2002), hence it is very likely that both these mechanisms work in concert to protect against the psychotogenic effects of Δ9-THC. As mentioned previously, CBD has many more pharmacological targets which may also contribute to its effects.These include activation of GPR55 (Ryberg et al., 2007), inhibition of adenosine uptake (Carrier et al., 2006), inhibition of VDAC1 (Rimmerman et al., 2013), transient receptor potential ion cannel and 5-HT_{1A} agonism(McPartland et al., 2014), among others. However, further studies are needed to elucidate the role of these targets in relation to CBDs anti-psychotic effects.

The most consistently reported negative effects of cannabis intoxication are impairments to cognitive processes. Previous intravenous studies have reported impairments to immediate and delayed verbal recall, executive function and working memory, while not affecting verbal fluency and learning (D'Souza et al., 2004; Morrison et al., 2009). These studies administered relatively high doses, 2.5mg and 5mg of IV Δ9-THC to healthy volunteers, while the doses given in this thesis were 1.5mg and 1mg. The dose of 1.5mg Δ9-THC impaired working memory, immediate and delayed verbal recall, while not affecting executive function and speed of processing. Relatively, 1mg Δ9-THC impaired delayed verbal recall, while not affecting immediate recall or working memory. Taken together, these results suggest that delayed verbal recall is the most susceptible cognitive domain as this was still negatively impacted by

the lowest IV dose of Δ9-THC given. This was followed in order by: working memory (digit span, first reverse then forward), immediate verbal recall, executive function, and not significantly affecting learning, speed of processing or verbal fluency. Other studies have also reported executive functioning being less impaired by cannabis (Pope et al., 1995; Ramaekers et al., 2006b), whist one study found that cannabis improved verbal fluency among participants classified as low-creative (Schafer et al., 2012).

In this thesis, I have shown that both CBD and Δ9-THCV protected the most vulnerable of cognitive domains, delayed recall, against the effects of Δ9-THC. Interestingly, CBD was unable to protect against the impairing effects of Δ9-THC on digit span or immediate recall, while both these domains were unaffected by a lower dose of Δ9-THC. This suggests that the protective effects of CBD are highly specific, and that CBD might not be able to protect against Δ9-THCs effects on working memory if the dose is too high. In a naturalistic study where participants smoked their own cannabis, Morgan and colleagues found that participants who smoked cannabis with higher levels of CBD did not show any impairment to both immediate and delayed recall whist intoxicated (Morgan, Schafer, et al., 2010). However, in that study the participants were asked to smoke as they normally would to reach their desired level of intoxication, while in the studies for this thesis the Δ9-THC doses were fixed.

The EEG findings of this thesis are somewhat mixed compared to previous research on the effects of cannabis or Δ9-THC on electrophysiological measures. Most studies to date have reported reductions to theta, alpha and beta power following administration of Δ9-THC (Ilan et al., 2004, 2005; Koen B E Böcker et al., 2010). In these studies reduction to EEG power were related to impairments to cognitive performance. Morrison and colleagues administered 1.25mg IV Δ9-THC to healthy volunteers and observed a significant reduction in theta power and coherence (Morrison et al., 2011). Alpha power was reduced at trend-level, while the other frequency bands did not significantly change. They found that the reduction in bi-frontal theta coherence was significantly correlated with positive psychotic symptoms. In the current study, I replicated these results in that theta amplitude and coherence were significantly reduced following Δ9-THC, although the reduction in theta coherence was not correlated with psychotic symptoms. Furthermore, unlike previous studies beta and delta amplitude increased following Δ9-THC, and alpha amplitude increased after each

subsequent testing session. Interestingly, CBD significantly inhibited Δ9-THC-induced delta increase. However, as Δ9-THC-induced delta increase was not hypothesised a priori, correlations between this and psychotic symptoms were not performed. A potential explanation for the discrepant findings of this study is that all three recording sessions took place on the same day, while previous studies studied participants on separate occasions (Koen B E Böcker et al., 2010; Ilan et al., 2004, 2005; Morrison et al., 2011). Due to the long experimental day, which included multiple sessions of cognitive testing, participant fatigue might have interacted with the pharmaceuticals given to produce the results reported here.

Implications

This research has potential importance to public health as well as for mental illness. As previously discussed, cannabis products are becoming more potent, with growers specifically breeding plants high in Δ9-THC at the expense of reduced CBD content. People who chose to smoke cannabis today will mostly be exposed to stronger cannabis products which may be more harmful to their mental health (Di Forti et al., 2009), cognition (Morgan, Schafer, et al., 2010), and hold a greater risk of addiction (Morgan, Freeman, et al., 2010). Heavy cannabis users, who are more tolerant towards the effects of cannabis and require higher amounts of Δ9-THC compared to infrequent users (Hirvonen et al., 2012; D'Souza, Ranganathan, et al., 2008), will be at even greater risk to the negative effects of cannabis.

Another source of harm which has recently emerged are in the form of synthetic cannabinoid agonists, known commonly as "Spice" (Spaderna et al., 2013). These products have recently become increasingly popular as many of them are still not illegal and are not detectable by standard urine drug screens. However, many of these cannabinoids are far more potent than Δ9-THC and produce a higher prevalence of adverse effects (Winstock and Barratt, 2013). A recent report found that admission to emergency services was 30 times more likely for people using spice compared to regular cannabis (Winstock, 2013).

The risk of cannabis use to recreational users could potentially be reduced by the concomitant use of other cannabinoids such as CBD and Δ9-THCV. This might be achieved by either promoting the production of CBD and Δ9-THCV high strains of

cannabis, or alternatively prescribing these cannabinoids to frequent users. Furthermore, if cannabis use is causally related to the development of psychotic illness such as schizophrenia, the presence of CBD may prevent certain individuals from developing the disorder.

Cannabinoids such as CBD may also hold therapeutic potential in treating psychotic disorders. So far, naturalistic, observational and experimental studies have repeatedly demonstrated the ameliorating effects of CBD against the psychotogenic effects of Δ9-THC in healthy volunteers. To date, only one clinical trial has shown the efficacy of CBD in treating schizophrenia (Leweke et al., 2012). If more studies confirm these findings, patients could benefit from an effective anti-psychotic medication with very few and mild side effects (sedation at higher doses). Furthermore, it may also benefit some patients who have not responded to regular anti-psychotic treatment, as CBD exerts its effects via a different pharmacological pathway than the classic dopaminergic one.

Lastly, cannabinoid receptor antagonists such as Δ9-THCV may show therapeutic benefits for various metabolic conditions. The CB1 inverse agonist Rimonabant showed great efficacy in decreasing weight and improving cholesterol levels in obese patients (Christensen et al., 2007), although it was withdrawn from the market due to psychiatric side-effects. Therefore, a CB1 antagonist which does not induce inverse agonism, such as Δ9-THCV, could potentially be effective in treating these metabolic measures while having a less negative side-effect profile.

Future directions

As demonstrated in this thesis, lower doses of Δ9-THC may not induce cognitive impairment and psychotic symptoms. Future studies would benefit from employing a within-subject design (to eliminate inter-individual variability) to explore a potential threshold dose of Δ9-THC which does not produce these negative effects. Furthermore, these studies could administer varying doses of both CBD and Δ9-THC to potentially find an optimal ratio between the two cannabinoids. Also, the combination of both CBD and Δ9-THCV could have a synergistic beneficial effect against the effects of Δ9-THC, compared to either cannabinoid administered individually.

In this thesis the results of the EEG analyses were somewhat contrary to previous studies. Further studies are needed to replicate these findings, preferably testing participants on separate days. Also, studying the EEG effects of a wider dose range of IV Δ9-THC might elucidate at which dose Δ9-THC begins to disrupt neural networks. Similarly, co-administration of different CBD and Δ9-THCV doses would also add to the understanding of how these cannabinoids protect neural networks against Δ9-THC.

Lastly, future studies should further explore the anti-psychotic effects of CBD. These studies should aim to treat patients suffering from different forms of psychotic illness, preferably with CBD as their sole treatment versus regular anti-psychotic medication. Alternatively, studies could explore the benefits of CBD as an addition to regular anti-psychotic medication, for either first episode patients or those who have been resistant to other treatments.

Publications pending

The 3rd and 4th chapters of this thesis are currently in preparation and will soon be submitted for publication.

Appendix

I.

PANSS

1-absent/does not apply

2-minimal

3-mild

4-moderate

5-moderate severe

6-severe

7-extreme

P1 Delusions	1	2	3	4	5	6	7
P2 Conceptual Disorganisation	1	2	3	4	5	6	7
P3 Hallucinatory Behaviour	1	2	3	4	5	6	7
P4 Excitement	1	2	3	4	5	6	7
P5 Grandiosity	1	2	3	4	5	6	7
P6 Suspiciousness/persecution	1	2	3	4	5	6	7
P7 Hostility	1	2	3	4	5	6	7
N1 Blunted Affect	1	2	3	4	5	6	7
N2 Emotional Withdrawal	1	2	3	4	5	6	7
N3 Poor Rapport	1	2	3	4	5	6	7
N4 Passive/apathetic social withdrawal	1	2	3	4	5	6	7
N5 Difficulty in abstract thinking	1	2	3	4	5	6	7
N6 Lack of spontaneity & flow of conversation	1	2	3	4	5	6	7
N7 Stereotyped Thinking	1	2	3	4	5	6	7
G1 Somatic Concern	1	2	3	4	5	6	7
G2 Anxiety	1	2	3	4	5	6	7
G3 Guilty Feelings	1	2	3	4	5	6	7
G4 Tension	1	2	3	4	5	6	7
G5 Mannerisms and posturing	1	2	3	4	5	6	7

G6 Depression	1	2	3	4	5	6	7
G7 Motor Retardation	1	2	3	4	5	6	7
G8 Uncooperativeness	1	2	3	4	5	6	7
G9 Unusual Thought Content	1	2	3	4	5	6	7
G10 Disorientation	1	2	3	4	5	6	7
G11 Poor Attention	1	2	3	4	5	6	7
G12 Lack of judgement and insight	1	2	3	4	5	6	7
G13 Disturbance of Volition	1	2	3	4	5	6	7
G14 Poor impulse control	1	2	3	4	5	6	7
G15 Pre-occupation	1	2	3	4	5	6	7
G16 Active social avoidance	1	2	3	4	5	6	7

II.

SSPS (BASELINE)

We are interested in your views. Please circle **how much you agree or disagree** with the following statements based upon your thoughts in the last 15-20 minutes.

	Do not agree	Agree a little	Agree moderately	Agree very much	Totally agree
1. Someone was hostile towards me	1	2	3	4	5
2. No-one had any particular feelings about me	1	2	3	4	5
3. Someone had bad intentions towards me	1	2	3	4	5
4. Someone was friendly towards me	1	2	3	4	5
5. Someone was trying to make me distressed	1	2	3	4	5
6. I felt very safe in their company	1	2	3	4	5
7. Someone stared at me in order to upset me	1	2	3	4	5
8. Everyone was trustworthy	1	2	3	4	5
9. Someone wanted me to feel threatened	1	2	3	4	5
10. I wasn't really noticed by anybody	1	2	3	4	5
11. Someone had kind intentions toward me	1	2	3	4	5
12. Someone would have harmed me in some way if they could	1	2	3	4	5
13. Someone had it in for me	1	2	3	4	5

14. Everyone was neutral towards me	**1**	**2**	**3**	**4**	**5**
15. Someone was trying to intimidate me	**1**	**2**	**3**	**4**	**5**
16. Everyone was pleasant	**1**	**2**	**3**	**4**	**5**
17. Someone was trying to isolate me	**1**	**2**	**3**	**4**	**5**
18. No-one had any intentions towards me	**1**	**2**	**3**	**4**	**5**
19. Everyone seemed unconcerned by my presence	**1**	**2**	**3**	**4**	**5**
20. Someone was trying to irritate me	**1**	**2**	**3**	**4**	**5**

III.

UMACL

Does the adjective describe your mood in the last 15-20 minutes?

.

	Definitely	Slightly	Slightly not	Definitely not
Happy	1	2	3	4
Dissatisfied	1	2	3	4
Energetic	1	2	3	4
Relaxed	1	2	3	4
Alert	1	2	3	4
Nervous	1	2	3	4
Passive	1	2	3	4
Cheerful	1	2	3	4
Tense	1	2	3	4
Jittery	1	2	3	4
Sluggish	1	2	3	4
Sorry	1	2	3	4
Composed	1	2	3	4
Depressed	1	2	3	4
Restful	1	2	3	4

Vigorous	1	2	3	4
Anxious	1	2	3	4
Satisfied	1	2	3	4
Un-enterprising	1	2	3	4
Sad	1	2	3	4
Calm	1	2	3	4
Active	1	2	3	4
Contented	1	2	3	4
Tired	1	2	3	4

IV.

Form 1

Semantic Categories: Four-Legged Animals, Precious Stones, Human Dwellings

Name _____ Sex _____ Age _____ years _____ months

Examiner _____ Date ____/____/____

Word List	Learning Trials			Delayed Recall Trial (20-25 min.)
	Trial 1	Trial 2	Trial 3	Trial 4
LION				
EMERALD				
HORSE				
TENT				
SAPPHIRE				
HOTEL				
CAVE				
OPAL				
TIGER				
PEARL				
COW				
HUT				
Total correct responses =				

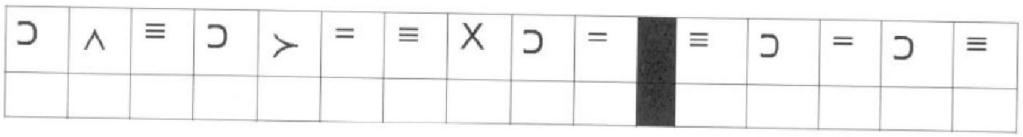
Completion Time Trial 3 _____ Start Time Trial 4 _____

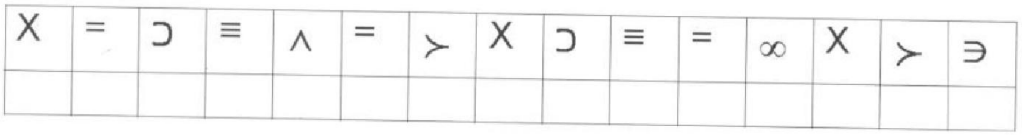
3

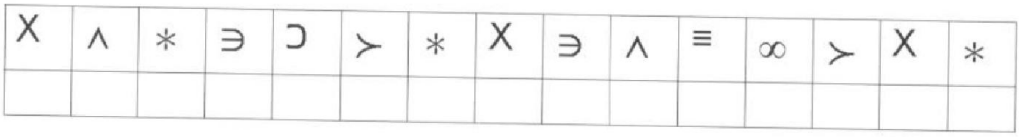
V.

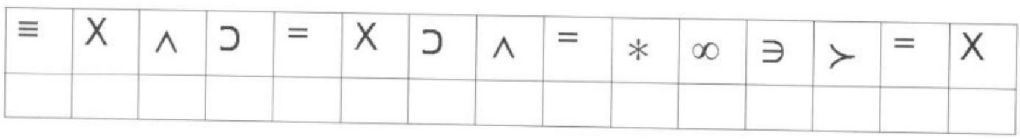
KEY

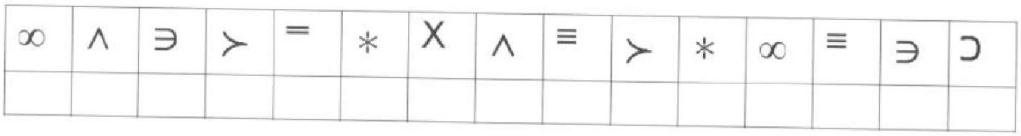
⊃	≡	≻	X	∧	=	*	∋	∞
1	2	3	4	5	6	7	8	9

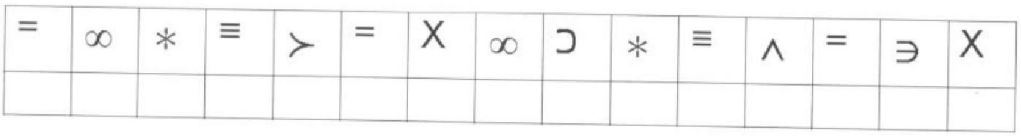


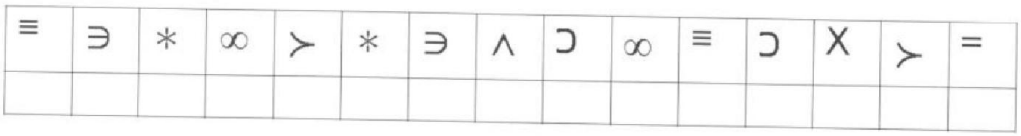


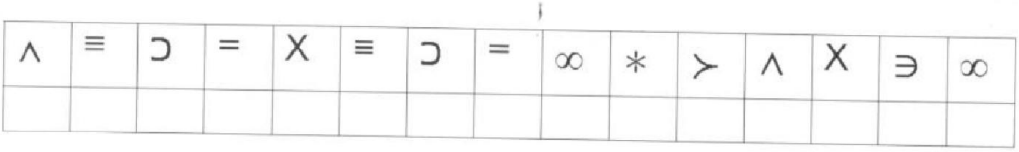












VI.

Digit Span

Instructions: I am going to say a list of numbers. When I am finished I want you to tell me the numbers in the order in which they were given.

Rules: If successful, move on immediately to the next level. Two attempts at each level of difficulty. If wrong x 2 then stop and score.

Name Date Code

Forward Span

2-9

7-1

3-6-5

2-4-9

3-1-7-4 *start*

4-6-2-9

1-8-5-2-4

8-7-1-9-5

2-4-7-3-9-1

1-9-5-7-4-3

5-6-3-9-2-1-8

6-4-3-2-8-5-1

2-7-5-8-6-4-9-3

9-4-3-7-6-2-5-8

7-4-5-8-4-7-9-3-1

3-2-6-5-3-7-8-9-2

5-4-6-8-2-6-3-2-7-9

2-4-5-8-5-8-3-7-1-4

Score 

Instructions: Again, I am going to say a list of numbers. This time I want you to tell me the numbers in the reverse order.

Reversed Span

2-9

9-4

7-8-2 ***start***

5-8-1

7-8-6-4

8-4-1-7

8-2-5-9-4

5-8-6-3-9

9-2-4-8-7-1

3-7-4-9-1-6

8-7-5-2-6-3-9

4-8-1-2-5-9-7

5-9-4-6-5-3-8-7

9-4-6-2-8-3-7-5

6-2-4-6-7-3-8-9-5

7-3-5-7-5-3-2-8-4

2-4-1-5-6-3-6-7-4-7

4-2-5-7-5-8-9-3-2-6

Score

VII.

Recording	Scoring	Discontinuation
Place a check mark (✓) to indicate whether or not the maze was completed within time limit. Record completion time in seconds.	Award 0 points if examinee does not complete the maze within the time limit. If examinee successfully completes the maze within the time limit, circle the appropriate score that corresponds to the completion time for that maze.	Discontinue after three consecutive scores of 0 points.

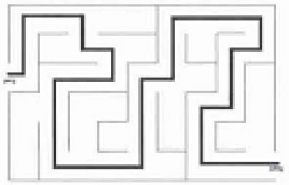
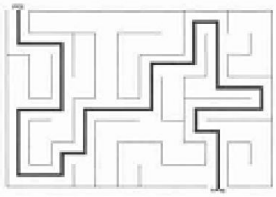
Administration Instructions

Say, I am going to give you some mazes to complete. Open the Mazes Test Response Booklet to Maze A and say, I want you to work as quickly as you can to complete this maze. Try your best not to make any errors or stray marks. Here is the "start" (point) where you will begin and here is the "end" (point) where you will finish. You may not cut corners or cross over any lines to reach the end. Also, I do not want you to lift your pen once you have started the maze. Hand pen to examinee. Ready? BEGIN. Begin timing. Allow examinee to complete Maze A. If examinee makes several mistakes, demonstrate correct completion using a different colored pen. For Mazes B through G, say, Here is another maze. Start here (point) and end here (point). Remember not to lift your pen once you have made a mark. Ready? BEGIN. Begin timing.

If examinee begins any place other than "Start," stop and redirect immediately. If examinee asks whether he/she can self-correct, say, Yes, you can cross back over your own lines, but do not lift your pen.

If examinee stops at a dead end, say, Keep trying and see if you can work your way out. If he/she refuses, record time and award a score of 0. An error is defined as crossing over any line by more than ¼ inch. If an error occurs on a straight line, stop examinee and mark the error by making a slash through the line, then direct him/her to the point of the error and instruct him/her to continue from that point. On a corner, an error is defined as cutting that corner so that the corner is now rounded and the cut is greater than ¼ inch away from the vertex. Again, stop examinee and mark the error by making a slash through the line, then direct him/her to the point of the error and instruct him/her to continue from that point. Do not allow the examinee to rotate the maze.

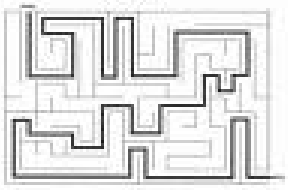
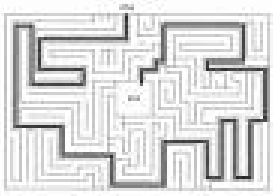
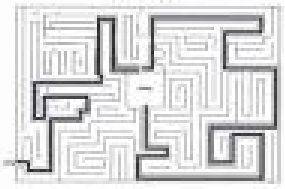
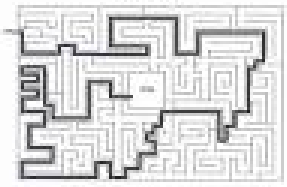
EXAMINEE

Maze Item	Time Limit	Completed	Completion Time (in seconds)	Score
Maze A 	30 sec.	☐ No		0
		☐ Yes		2 (1-3 sec.) 1 (4-30 sec.)
Maze B 	30 sec.	☐ No		0
		☐ Yes		2 (1-11 sec.) 1 (12-30 sec.)
Maze C 	30 sec.	☐ No		0
		☐ Yes		2 (1-15 sec.) 1 (16-30 sec.)

EXAMINER

Continue →

EXAMINEE

Maze Item	Time Limit	Completed	Completion Time (in seconds)	Score
Maze D 	120 sec.	☐ No		0
		☐ Yes		5 (1-32 sec.) 4 (33-45 sec.) 3 (46-59 sec.) 2 (60-79 sec.) 1 (80-120 sec.)
Maze E 	240 sec.	☐ No		0
		☐ Yes		5 (1-73 sec.) 4 (74-100 sec.) 3 (101-126 sec.) 2 (127-164 sec.) 1 (165-240 sec.)
Maze F 	240 sec.	☐ No		0
		☐ Yes		5 (1-87 sec.) 4 (88-119 sec.) 3 (120-146 sec.) 2 (147-184 sec.) 1 (185-240 sec.)
Maze G 	240 sec.	☐ No		0
		☐ Yes		5 (1-89 sec.) 4 (90-129 sec.) 3 (130-168 sec.) 2 (169-201 sec.) 1 (202-240 sec.)
EXAMINER				Mazes (MAZ) Raw Score

Qualitative Features (✓ if present)

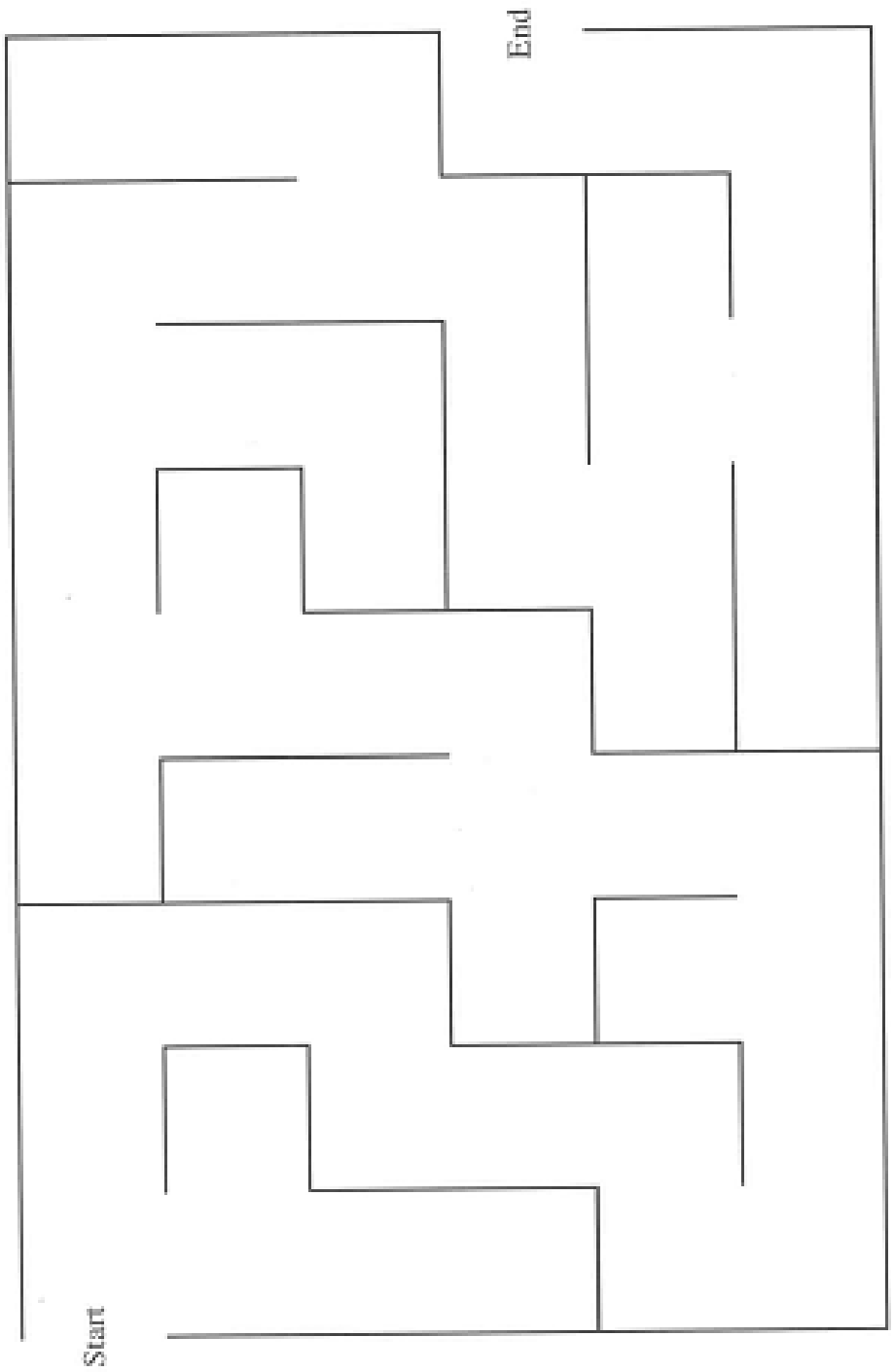
☐ Long latency before beginning mazes ☐ Impulsive/quick start ☐ Haphazard approach ☐ Crossing line errors

Comments/Notes:

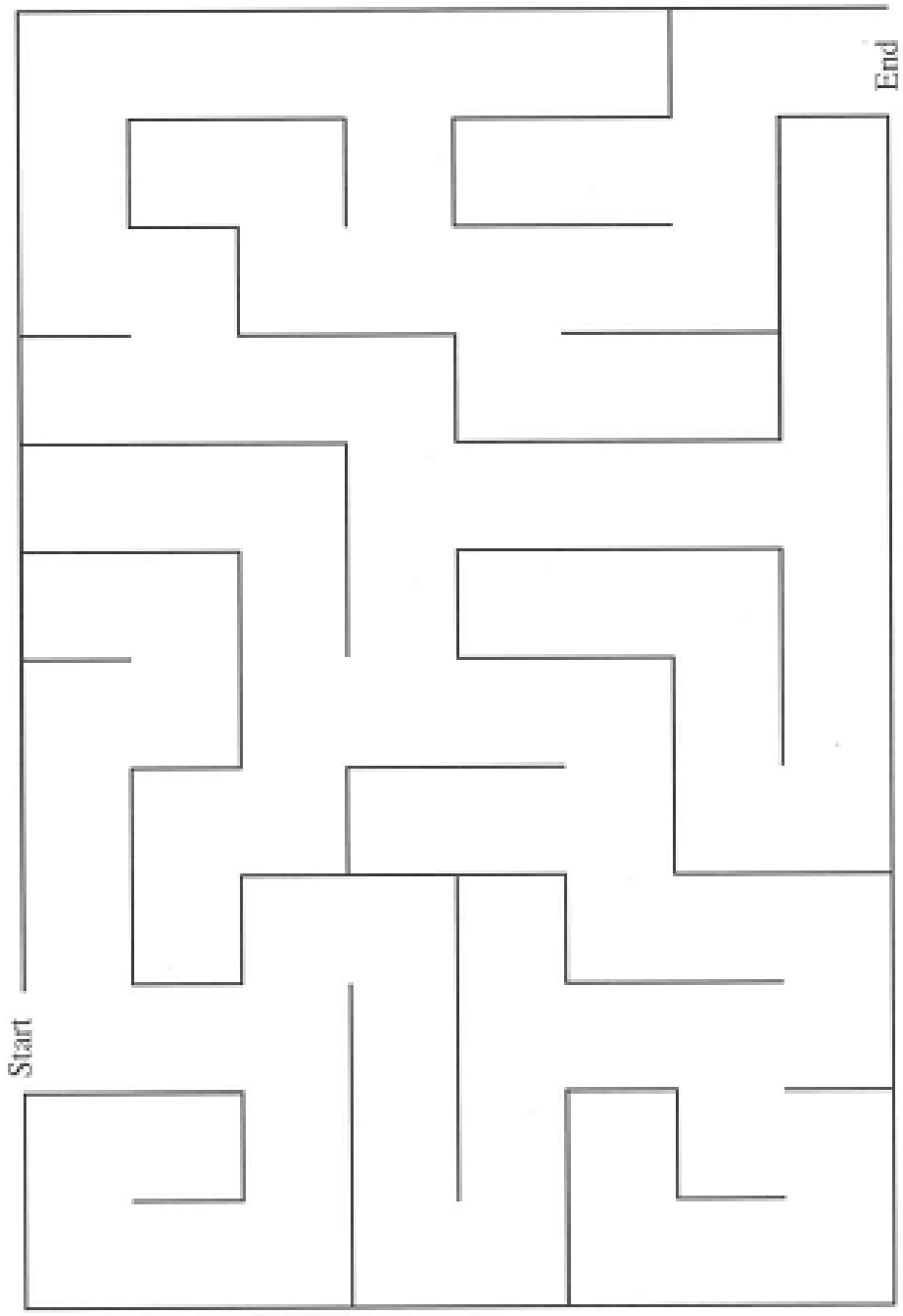
3

A

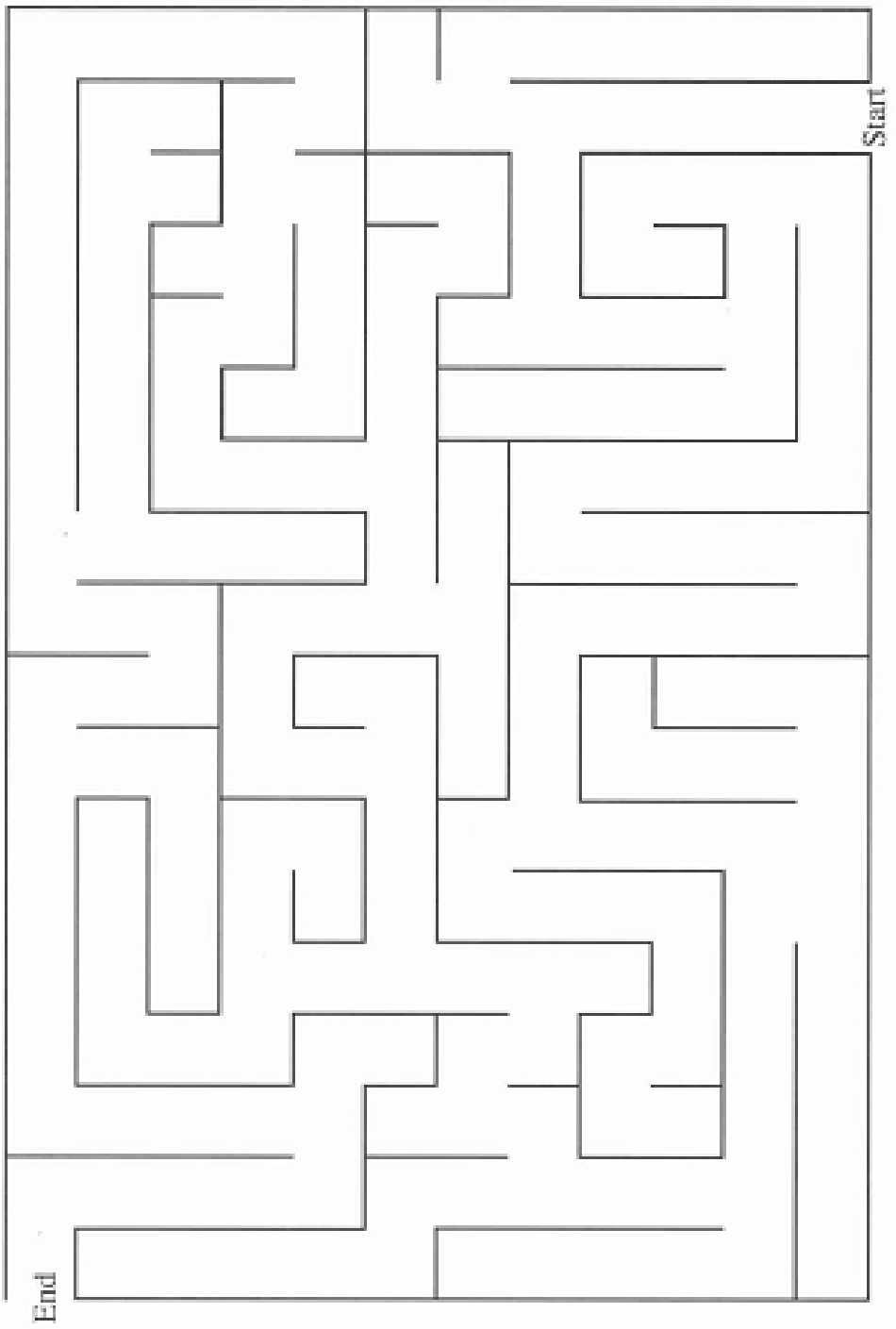
B



c

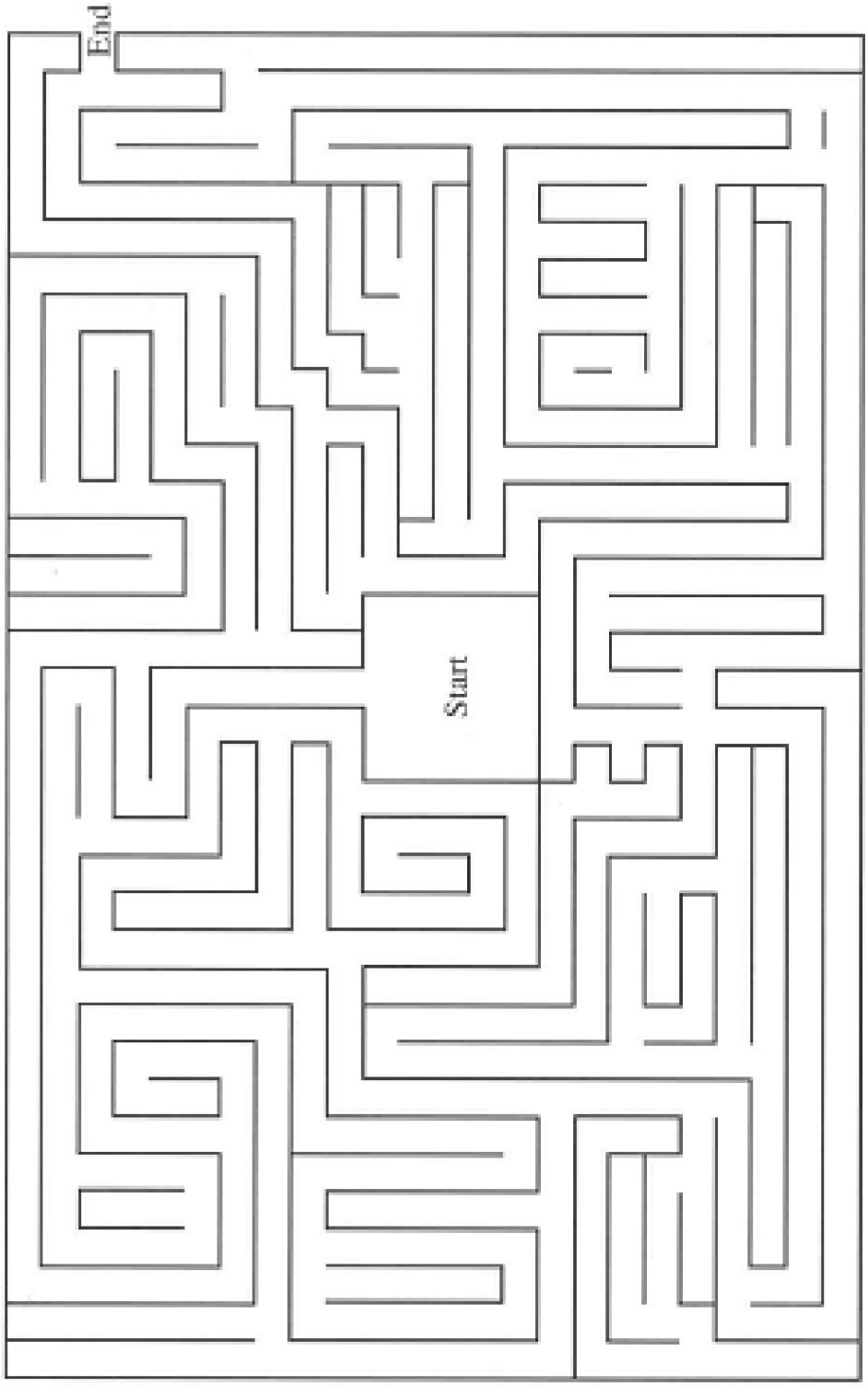


D

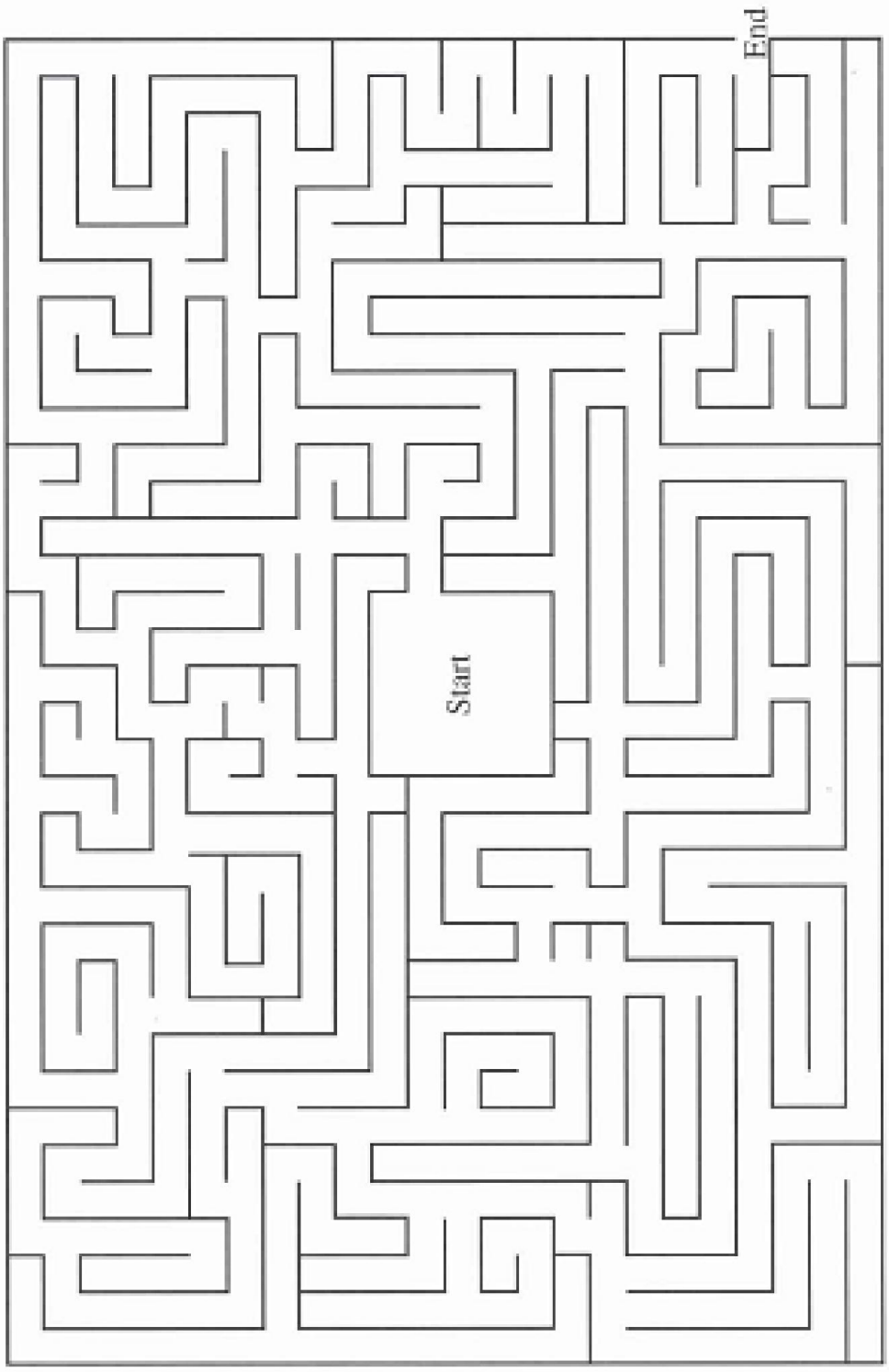


E

F



G



VIII.

0Back	**D**	**1Back**	**E**	**3Back**	**D**
0Back	**F**	**1Back**	**Q**	**3Back**	**W**
0Back	**U**	**1Back**	**T**	**3Back**	**D**
0Back	**X**	**Instr Two back**		**3Back**	**S**
0Back	**P**	**2Back**	**D**	**3Back**	**E**
0Back	**X**	**2Back**	**B**	**3Back**	**C**
0Back	**N**	**2Back**	**C**	**3Back**	**U**
0Back	**A**	**2Back**	**R**	**3Back**	**S**
0Back	**M**	**2Back**	**S**	**3Back**	**C**
0Back	**R**	**2Back**	**R**	**Instr Is it 'X'**	
0Back	**S**	**2Back**	**J**	**0Back**	**J**
0Back	**X**	**2Back**	**U**	**0Back**	**G**
0Back	**W**	**2Back**	**H**	**0Back**	**D**
0Back	**O**	**2Back**	**Y**	**0Back**	**M**
Instr One back		**2Back**	**P**	**0Back**	**X**
1Back	**F**	**2Back**	**Y**	**0Back**	**V**
1Back	**S**	**2Back**	**W**	**0Back**	**N**
1Back	**R**	**2Back**	**B**	**0Back**	**B**
1Back	**R**	**Instr Three back**		**0Back**	**X**
1Back	**C**	**3Back**	**T**	**0Back**	**H**
1Back	**W**	**3Back**	**S**	**0Back**	**P**
1Back	**V**	**3Back**	**M**	**0Back**	**F**
1Back	**V**	**3Back**	**I**	**0Back**	**I**
1Back	**D**	**3Back**	**J**	**0Back**	**X**
1Back	**A**	**3Back**	**M**	**Instr Three back**	
1Back	**A**	**3Back**	**P**	**3Back**	**R**

3Back P
3Back W
3Back E
3Back G
3Back F
3Back E
3Back L
3Back T
3Back R
3Back S
3Back V
3Back F
3Back E
3Back V
3Back W

Instr Two back

2Back G
2Back B
2Back C
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2Back O
2Back R
2Back J
2Back R
2Back P
2Back M
2Back Y
2Back M
2Back W
2Back V

Instr One back

1Back F
1Back R
1Back S
1Back S
1Back T
1Back W
1Back C
1Back Z
1Back D
1Back E
1Back E
1Back A
1Back Q
1Back T

Instr Is it 'X'

0Back G
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IX.

CAPE-state (Cannabis)

The CAPE-state is designed to measure feelings, thoughts and mental experiences. We believe that these are much more common than has previously been supposed, and that most people have had some such experiences during their lives.
The next pages are divided into columns A and B. Please use column A to indicate if you are experiencing the specific feelings, thoughts or mental experiences **at this time.** Please answer the following questions as honestly as you can. **There are no right or wrong answers and there are no trick questions.**

If you answered **'yes** to the question in **column A**, please circle the number in **column B that corresponds most closely to how distressing these feelings, thoughts or experiences are to you.** After this, **go to the next question in column A.**
If you answered **no** to the **question in column A**, don't answer the matching question in column B, but **go straight to the next question in column A**.

If *yes* in column A, go to column B. If *no* in column A, go straight to the next question in column A.

Column A	**Column B**
Do you have a specific feeling, thought or mental experience in the last 10 minutes?	**How distressed are you by this experience?**

ID-no: [redacted]						
			not distressing	a bit distressing	quite distressing	very distressing
1) Do you feel sad?	no	yes	0	1	2	3
2) Do you feel as if people seemed to be dropping hints about you or saying things with a double meaning?	no	yes	0	1	2	3
3) Do you feel that you are not very animated?	no	yes	0	1	2	3
4) Do you feel that you are not much of a talker at the moment?	no	yes	0	1	2	3
5) Do you feel as if things in magazines or newspapers were written especially for you?	no	yes	0	1	2	3
6) Do you feel as if some people were not what they seemed to be?	no	yes	0	1	2	3
7) Do you feel as if you are being persecuted in some way?	no	yes	0	1	2	3
8) Do you feel that you experience few or no emotions at this time?	no	yes	0	1	2	3
9) Do you feel pessimistic about everything?	no	yes	0	1	2	3
10) Do you feel as if there is a conspiracy against you?	no	yes	0	1	2	3
11) Do you feel as if you are destined to be someone very important?	no	yes	0	1	2	3

12) Do you feel as if there is no future for you?	no	yes	0	1	2	3
13) Do you feel that you are a very special or unusual person?	no	yes	0	1	2	3
14) Do you feel as if you do not want to live anymore?	no	yes	0	1	2	3

ID-no:						
			not distessing	a bit distressing	quite distressing	very distressing
15) Do you think that people can communicate telepathically?	no	yes	0	1	2	3
16) Do you have no interest in being with other people?	no	yes	0	1	2	3
17) Do you feel as if electrical devices such as computers can influence the way you are thinking?	no	yes	0	1	2	3
18) Do you lack motivation to do things?	no	yes	0	1	2	3
19) Are you crying about nothing?	no	yes	0	1	2	3
20) Do you feel the power of witchcraft, voodoo or the occult?	no	yes	0	1	2	3
21) Do you feel that you are lacking in energy?	no	yes	0	1	2	3
22) Do you feel that people are looking at you oddly?	no	yes	0	1	2	3
23) Do you feel that your mind is empty?	no	yes	0	1	2	3
24) Do you feel as if the thoughts in your head are being taken away from you?	no	yes	0	1	2	3
25) Do you feel that you are spending your time doing nothing?	no	yes	0	1	2	3
26) Do you feel as if the thoughts in your head are not your own?	no	yes	0	1	2	3
27) Do you feel that your feelings are lacking in intensity?	no	yes	0	1	2	3
28) Are your thoughts so vivid that you are worried other people might hear them?	no	yes	0	1	2	3
29) Do you feel you are lacking in spontaneity?	no	yes	0	1	2	3
30) Do you hear your own thoughts being echoed back to you?	no	yes	0	1	2	3
[redacted]						

ID-no:	[redacted]

			not distressing	a bit distressing	quite distressing	very distressing
31) Do you feel as if you are under the control of some force or power other than yourself?	no	yes	0	1	2	3
32) Do you feel that your emotions are blunted?	no	yes	0	1	2	3
33) Do you hear voices that other people could not hear?	no	yes	0	1	2	3
34) Do you hear voices talking to each other that other people could not hear?	no	yes	0	1	2	3
35) Do you feel that your appearance or personal hygiene are no longer important?	no	yes	0	1	2	3
36) Do you feel that you cannot get things done?	no	yes	0	1	2	3
37) Do you feel that few things are of interest to you?	no	yes	0	1	2	3
38) Do you feel guilty about something?	no	yes	0	1	2	3
39) Do you feel like a failure?	no	yes	0	1	2	3
40) Do you feel tense?	no	yes	0	1	2	3
41) Do you feel as if a double has taken the place of one of the people you are in the room with?	no	yes	0	1	2	3
42) Do you see objects, people or animals that other people cannot see?	no	yes	0	1	2	3

X.

BAI

Below is a list of common symptoms. Indicate how much you have been bothered by that symptom in the last 15-20 minutes.

.

	Not At All	Mildly but it didn't bother me much	Moderately - it wasn't pleasant at times	Severely – it bothered me a lot
Numbness or tingling	0	1	2	3
Feeling hot	0	1	2	3
Wobbliness in legs	0	1	2	3
Unable to relax	0	1	2	3
Fear of worst happening	0	1	2	3
Dizzy or lightheaded	0	1	2	3
Heart pounding/racing	0	1	2	3
Unsteady	0	1	2	3
Terrified or afraid	0	1	2	3
Nervous	0	1	2	3
Feeling of choking	0	1	2	3
Hands trembling	0	1	2	3
Shaky / unsteady	0	1	2	3
Fear of losing control	0	1	2	3
Difficulty in	0	1	2	3

breathing				
Fear of dying	0	1	2	3
Scared	0	1	2	3
Indigestion	0	1	2	3
Faint / lightheaded	0	1	2	3
Face flushed	0	1	2	3
Hot/cold sweats	0	1	2	3

Column Sum

Scoring - Sum each column. Then sum the column totals to achieve a grand score. Write that

score here _____ .

XI.

Visual Analogue Scale

High: Giggly, full of life, mischievous, playful, seeing the funny side of things, interested in others, the situation, percepts and ideas, light, cheerful, word-play, seeing connections, jocular, creative.

"Feeling high"

0 10

_____

Not high at all As high as could possibly be

Anxious: Feeling fearful and panicky, anticipating something bad to happen with dry mouth, muscles tension, hand/knee shakes, sweating, shortness of breath, dizziness, butterflies in stomach and nausea.

“Feeling anxious”

0 10

Not anxious at all As anxious as could possibly be

Tired: Lacking in energy, feeling drained, wanting to lie down.

“Feeling tired”

0 10

Not tired at all As tired as could possibly be

Calm and relaxed: Feeling at peace, with no muscle tension.

"Feeling calm and relaxed"

0 10

_____

Not calm and relaxed at all As calm and relaxed as could possibly be

Stoned: Slowed up, introverted, reduced capacity for thought, movement and emotion, feeling less reactive, sleepy, heavy and dull. Disinterest in others and the surroundings. Closed off.

"Feeling stoned"

0 10

_____

Not stoned at all As stoned as could possibly be

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